UNIWERSYTET PRZYRODNICZY W POZNANIU

WYDZIAŁ NAUK O ŻYWNOŚCI I ŻYWIENIU

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WARTOŚĆ ODŻYWCZA I WŁAŚCIWOŚCI PROZDROWOTNE ŻYWNOŚCI NISKOPRZETWORZONEJ W POSTACI SKIEŁKOWANYCH NASION GRYKI *FAGOPYRUM ESCULENTUM* MOENCH W OBECNOŚCI DROŻDŻY PROBIOTYCZNYCH *SACCHAROMYCES CEREVISIAE* VAR. *BOULARDII*

Praca doktorska wykonana

w Katedrze Żywienia Człowieka i Dietetyki,

pod kierunkiem

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PODZIĘKOWANIA

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STRESZCZENIE

Wartość odżywcza i właściwości prozdrowotne żywności niskoprzetworzonej w postaci skiełkowanych nasion gryki *Fagopyrum esculentum* Moench w obecności drożdży probiotycznych *Saccharomyces cerevisiae* var. *boulardii*

Gryka należy do rodziny *Polygonaceae* i jest określana jako „pseudozboże", ponieważ przypomina zboża pod względem składu chemicznego oraz możliwości zastosowania nasion podobnych do ziaren zbóż. Najpopularniejszymi odmianami są gryka zwyczajna (*Fagopyrum esculentum* Moench) i gryka tatarka (*Fagopyrum tataricum* (L.) Gaertn.). Główną formą gryki dostępną na rynku są nasiona, płatki śniadaniowe, mąki do wyrobów piekarniczych (m.in. chleba) oraz innych produktów wzbogaconych, jak również herbata, miód i kiełki. Ziarna omawianego pseudozboża zawierają różnorodne składniki odżywcze, z których najważniejszymi związkami są: białka, polisacharydy, błonnik pokarmowy, tłuszcze, polifenole, oraz składniki mineralne. Mają one wpływ na zdrowie człowieka i wykazują działanie hipotensyjne, hipoglikemiczne, hipocholesterolemiczne, neuroprotekcyjne, przeciwutleniające i przeciwnowotworowe.

Proces kiełkowania zmienia wartość odżywczą gryki i wpływa korzystnie m.in. na zawartość kwasów tłuszczowych, aminokwasów, cukrów redukujących, czy flawonoidów. Jednym ze sposobów zmiany wartości odżywczej surowca, a tym samym zmiany właściwości prozdrowotnych, jest modyfikacja surowca. Stąd też głównym celem pracy była ocena wpływu modyfikacji środowiska wzrostu gryki *Fagopyrum esculentum* Moench na wartość odżywczą i prozdrowotną otrzymanego surowca poprzez wprowadzenie, w trakcie kiełkowania, dodatku probiotycznych drożdży *Saccharomyces cerevisiae* var. *boulardii*. Modyfikacja środowiska wzrostu nasion gryki zwyczajnej, wpłynęła na zmiany wartości odżywczej kiełków. Zmiany zauważono, zarówno, w profilu czy ilości makroskładników, jak i w związkach bioaktywnych. Kiełki zmodyfikowane odznaczały się wyższą aktywnością antyoksydacyjną i przeciwzapalną, w porównaniu z kiełkami kontrolnymi. Wyniki uzyskane w ramach rozprawy doktorskiej wskazują na wielokierunkowy i zróżnicowany potencjał funkcjonalny zmodyfikowanych kiełków gryki zwyczajnej, dzięki czemu surowiec ten może być wykorzystany w przemyśle spożywczym, jako potencjalny dodatek do produktów spożywczych, szczególnie dla osób cierpiących z powodu przewlekłych chorób niezakaźnych.

Słowa kluczowe: gryka zwyczajna, probiotyczne drożdże, badania *in vitro,* badania *in vivo,* aktywność przeciwutleniająca, aktywność przeciwzapalna, biodostępność, strawność

ABSTRACT

Nutritional value and pro-health properties of low-processed food in the form of sprouted buckwheat seeds *Fagopyrum esculentum* Moench in the presence of probiotic yeast *Saccharomyces cerevisiae* var. *boulardii*

Buckwheat belongs to the family *Polygonaceae* and is referred to as a pseudocereal because it resembles cereals in terms of chemical composition and the possibility of using seeds similar to cereal grains. The most popular varieties are common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.). The main forms of buckwheat available on the market are seeds, breakfast cereals, flours for bakery products (including bread) and other enriched products, as well as tea, honey and sprouts. Buckwheat grains contain a variety of nutrients, the most important of which are: proteins, polysaccharides, dietary fibre, fats, polyphenols and minerals. They have an impact on human health and show hypotensive, hypoglycemic, hypocholesterolemic, neuroprotective, antioxidant and anticancer effects.

The germination process changes buckwheat's nutritional value and has a positive effect e.g. on the content of fatty acids, amino acids, reducing sugars or flavonoids. One of the ways to change the nutritional value of the raw material, and thus change the health-promoting properties, is to modify the raw material. Therefore, the main objective of the study was to assess the effect of modification of the growth environment of *Fagopyrum esculentum* Moench buckwheat on the nutritional and health-promoting value of the obtained raw material by introducing, during germination, the addition of probiotic yeast *Saccharomyces cerevisiae* var. *boulardii*. Modification of the growth environment of buckwheat seeds influenced the changes in the nutritional value of the sprouts. Changes were noticed in the profile or amount of macronutrients and in bioactive compounds. In addition, modified sprouts were characterized by higher antioxidant and anti-inflammatory activity compared to control sprouts. The results obtained as part of the doctoral dissertation indicate the multidirectional and diverse functional potential of modified buckwheat sprouts, thanks to which this raw material can be used in the food industry as a potential food additive, especially for people suffering from chronic non-communicable diseases.

Keywords: buckwheat, probiotic yeast, *in vitro* studies, *in vivo* studies, antioxidant activity, anti-inflammatory activity, bioavailability, digestibility

PUBLIKACJE WCHODZĄCE W SKŁAD ROZPRAWY DOKTORSKIEJ

P I.:

Molska, M.; Reguła, J.; Rudzińska, M.; Świeca, M. Fatty Acids Profile, Atherogenic and Thrombogenic Health Lipid Indices of Lyophilized Buckwheat Sprouts Modified with the Addition of *Saccharomyces Cerevisiae* Var. *Boulardii*. *Acta Scientiarum Polonorum Technologia Alimentaria* 2020, *19*, 483–490, http://doi.org/10.17306/J.AFS.2020.0866.

Punkty MNiSW: **40 pkt.**

P II.:

Molska, M.; Reguła, J.; Zielińska-Dawidziak, M.; Tomczak, A.; Świeca, M. Starch and Protein Analysis in Buckwheat (*Fagopyrum Esculentum* Moench) Sprouts Enriched with Probiotic Yeast. *LWT* 2022, 113903, https://doi.org/10.1016/j.lwt.2022.113903.

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P III.:

Molska, M.; Reguła, J.; Kapusta, I.; Świeca, M. Analysis of Phenolic Compounds in Buckwheat (*Fagopyrum Esculentum* Moench) Sprouts Modified with Probiotic Yeast. *Molecules* 2022, *27*, 7773, https://doi.org/10.3390/molecules27227773.

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P IV.:

Molska, M.; Reguła, J.; Świeca, M. Adding Modified Buckwheat Sprouts to an Atherogenic Diet – the Effect on Selected Nutritional Parameters in Rats. Plant Foods for Human Nutrition 2023, https://doi.org/10.1007/s11130-023-01047-9.

IF (2021): **4,124** Punkty MNiSW: **70pkt.**

P V.:

Molska, M.; Reguła, J.; Grygier, A.; Muzsik-Kazimierska, A.; Rudzińska, M.; Gramza-Michałowska, A. Effect of the Addition of Buckwheat Sprouts Modified with the Addition of *Saccharomyces Cerevisiae* Var. *Boulardii* to an Atherogenic Diet on the Metabolism of Sterols, Stanols and Fatty Acids in Rats. *Molecules* 2022, *27*, 4394, https://doi.org/10.3390/molecules27144394.

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SPIS TREŚCI:

ROZPRAWA DOKTORSKA

1. WPROWADZENIE

1.1. Charakterystyka gryki

Gryka pochodzi z Azji Środkowej i należy do rodziny rdestowatych. Obejmuje 21 gatunków, z których najpopularniejsze odmiany to gryka zwyczajna (*Fagopyrum esculentum* Moench) i gryka tatarka (*Fagopyrum tataricum* (L.) Gaertn.) [1–4]. W Europie pojawiła się w XIII wieku, dzięki ludom koczowniczym, najpierw w Austrii, Niemczech, Włoszech [1,5,6]. W 2020 roku światowa produkcja gryki wyniosła ponad 1,8 mln ton, natomiast w roku 2021 ponad 1,9 mln ton. Wiodącymi krajami w produkcji, we wskazanych latach według FAOSTAT były: Rosja, Chiny, Ukraina, Stany Zjednoczone Ameryki [1,7].

Gryka nie jest zbożem, ale ze względu na sposób jej uprawy i wykorzystania zwykle zaliczana jest do zbóż [8]. Należy do pseudozbóż, które w literaturze określa się, również jako „ziarna XXI wieku" lub „zboża rzekome". Pojęcie zboża rzekome odnosi się nasion, które pod względem funkcji i składu przypominają nasiona prawdziwych zbóż [1,9,10]. Pseudozboża, z botanicznego punktu widzenia są roślinami dwuliściennymi. Większość z nich daje wysokie plony i ma zdolność adaptacji do wymagających warunków środowiskowych [1–3]. W tabeli 1 została przedstawiona klasyfikacja botaniczna gryki.

Tabela 1. Botaniczna klasyfikacja gryki [6]

Klasa	*Dicotyledoneae*
Podklasa	*Caryophyllidae*
Rząd	*Polygonales*
Rodzina	*Polygonaceae*
Rodzaj	*Fagopyrum*
Wybrane gatunki	*F. tartaricum, F. esculentum* Moench

Gryka klasyfikowana jest jako tzw. roślina wtórna, co jest bezpośrednio związane z tym, że została otrzymana z chwastów obecnych, w innych uprawach [1,11]. Jedna roślina jest w stanie wyprodukować 500-2000 kwiatów, jednakże tylko 4-10% rozwija nasiona. Jest to spowodowane przede wszystkim warunkami pogodowymi w okresie wegetacji (zwłaszcza kwitnienia), a także odmianą. Gryka zwyczajna jest szczególnie wrażliwa na czynniki klimatyczne, nasłonecznienie, praktyki agrotechniczne, termin siewu. Jest rośliną

ciepłolubną, preferującą stanowiska słoneczne. Optymalna temperatura do kiełkowania to 15°C, a wzrostu 20°C. Niekorzystnie reaguje na mróz, długotrwałe susze oraz silne wiatry. Najlepiej plonuje na glebach złożonych pszenicznych o uregulowanym stosunku powietrze-woda oraz pH 5.6-7.0. Zapylana jest głównie przez pszczoły (tylko częściowo przez wiatr) [1-3].

W ostatnich latach, wzrasta zainteresowanie gryką, w związku z czym uzyskiwane są różne produkty piekarnicze i nie piekarnicze z dodatkiem gryki lub mąki gryczanej. Główną formą gryki dostępną na rynku są nasiona, natomiast produkty takie jak chleb, przekąski, kluski, herbatniki, ciastka, a także herbata, kiełki i miód, są obecnie komercjalizowane i coraz częściej spożywane. Wynika to ze znacznej poprawy jakości technologicznej oraz sensorycznej spowodowanej optymalizacją receptur i parametrów procesu technologicznego [1,10,11].

1.2. Kiełkowanie jako proces zmieniający wartość odżywczą surowca

W wielu krajach, kiełki uznawane są jako unikatowy surowiec, który cieszy się coraz większym zainteresowaniem ze strony konsumentów, wymagających od producentów żywności bez dodatków, naturalnej, minimalnie przetworzonej, o wysokiej wartości odżywczej [11]. Proces kiełkowania, znacznie podnosi wartość odżywczą poprzez zwiększenie biodostępności niektórych składników pokarmowych, np. witamin, czy składników mineralnych [1,9].

Przemiany biochemiczne i fizjologiczne, w następstwie których następują zmiany morfologiczne podczas kiełkowania, są silnie związane z przeżywalnością siewek i wzrostem wegetatywnym, które wpływają na plon i jego jakość. Ogólnie proces kiełkowania można podzielić na trzy fazy: faza I, szybkie wchłanianie wody przez nasiona; faza II, reaktywacja metabolizmu; i faza III, wysunięcie korzonków. Krytyczną fazą jest faza II, podczas której reaktywowane są podstawowe procesy fizjologiczne i biochemiczne, takie jak hydroliza, biosynteza makrocząsteczek, oddychanie, struktury subkomórkowe i wydłużanie komórek, co prowadzi do rozpoczęcia procesu kiełkowania [12–14].

Kiełki pseudozbóż są bogate w skrobię, białko oraz błonnik, jak również, są dobrym źródłem składników mineralnych (tj. wapń, cynk), witamin, fitochemikaliów (tj. saponiny, polifenole, fitosterole, betalaina), czyli związków o potencjalnie korzystnych właściwościach prozdrowotnych [1,10]. Proces kiełkowania jest istotnym etapem, który prowadzi do zmian w zawartości składników ziaren. Triacyloglicerole rozpoczynają proces hydrolizy i tym samym zwiększa się stosunek kwasów tłuszczowych nasyconych do nienasyconych. Zapasy skrobi są mobilizowane przez działanie α-amylazy, która powoduje zmianę ziarnistej powierzchni

i tworzenie dziur. Następują zmiany w składzie aminokwasowym białka. Ilość związków antyodżywczych (tj. fityniany, czy garbniki) znacznie się obniża, a zwiększeniu ulega ilość związków bioaktywnych np. fenoli [15–17]. Warto podkreślić, że związki zawarte w gryce wykazują szereg właściwości prozdrowotnych np. obniżanie poziomu cholesterolu w osoczu (białko gryki, czy flawonoidy), działają neuroprotekcyjnie (poprzez m.in. tyrozynazę, czy acetylocholinoesterazę) [18–21].

Moczenie i kiełkowanie to tradycyjne i najskuteczniejsze zabiegi mające na celu zwiększenie potencjału odżywczego i bioaktywnego oraz redukcję składników antyodżywczych w ziarnach [22]. W procesie kiełkowania ważna jest szybkość procesu, a następnie późniejsze wyniki wzrostu (w tym grubość hipokotylu, czy zmiany w związkach funkcjonalnych). Zauważa się potrzebę optymalizacji wskazanych czynników bowiem, z punktu widzenia wykorzystania ziarna, kiełkowanie jest jednym z bardzo istotnych procesów [23].

1.3. Proces kiełkowania, a mikroorganizmy

Kiełki mogą być biofortyfikowane w celu zwiększenia poziomu niezbędnych składników, a co za tym idzie, dodatkowej poprawy ich wartości odżywczej [24]. Można tego dokonać poprzez zaprawianie nasion mikroorganizmami, które okazały się korzystne dla roślin (środki kontroli biologicznej (BCA)) [25]. W publikacji Briatia i in. [8], zaszczepienie nasion bakterią endofityczną *Herbaspirillum* ST-B2 zwiększyło wzrost kiełków gryki zwyczajnej, sprzyjało wydłużaniu korzeni i zwiększało produkcję masową kiełków.

Potencjalnym czynnikiem, który, również, może wpłynąć na nasiona, a tym samym oddziaływać na jego właściwości są probiotyki. W badaniu Świeca i in. [26] autorzy wykazali, że kiełki roślin strączkowych wzbogacone o *Saccharomyces cerevisiae* var. *boulardii* stanowią nowy produkt funkcjonalny, charakteryzujący się zwiększonymi właściwościami zdrowotnymi i odżywczymi [26,27]. Według WHO probiotyki, w tym drożdże probiotyczne to „żywe szczepy ściśle wyselekcjonowanych mikroorganizmów, które podane w odpowiednich ilościach wywierają korzystny wpływ na zdrowie gospodarza” [28]. Mogą oddziaływać na jakość mikrobiologiczną oraz organoleptyczną żywności. Stąd też znajdują zastosowanie w produktach spożywczych m.in. mlecznych produktach fermentowanych [29].

Ponadto, kolejnym sposobem wykorzystania mikroorganizmów probiotycznych jest modyfikacja surowców, poprzez ich dodawanie. *Saccharomyces cerevisiae* var. *boulardii* jest zalecany jako czynnik biologiczny, który może być celowo dodawany do żywności lub paszy, zgodnie z notyfikacją EFSA 8 [30].

Wykazano, że kultury drożdży z różnych taksonów (przedstawiciele rodzajów m.in. *Aureobasidium, Cryptococcus, Debaryomyces, Sporobolomyces, Saccharomyces*) stymulują kiełkowanie nasion. W związku z tym pojawiła się sugestia, że mechanizm stymulujący kiełkowanie nasion obejmuje trzy etapy (wnikanie stymulantów do nasion, ich wpływ na mikroorganizmy endofityczne (aktywacja ich rozmnażania) oraz inicjowanie kiełkowania nasion przez mikroorganizmy endofityczne (ich produkty przemiany materii), a nie dwa etapy (przenikanie stymulantów do nasion i oddziaływanie na nie) [31]. W badaniu Mustafa i in. [32], wykazano, że moczenie nasion w ekstrakcie drożdżowym może zwiększyć podatność nasion na kiełkowanie.

1.4. Podsumowanie

Mikroorganizmy probiotyczne mogą wykazywać działanie prozdrowotne poprzez określone mechanizmy działania, takie jak efekty troficzne, regulacja immunologiczna i związane z nimi właściwości przeciwdrobnoustrojowe. *Saccharomyces cerevisiae* var. *boulardii* odznacza się szerokim zastosowaniem medycznym, które związane jest z profilaktyką biegunek poantybiotykowych, czy biegunek podróżnych; wydaje się również skutecznym w zapobieganiu nawrotom choroby u pacjentów z chorobą Leśniowskiego-Crohna [33–35]. Podczas choroby probiotyki są zwykle dostarczane do organizmu człowieka w postaci leków lub suplementów diety [36,37].

Gryka to doskonałe źródło składników odżywczych i związków o dobrze udokumentowanych właściwościach prozdrowotnych m.in. fenoli lub witamin [38,39]. Co ważne, gryka zawiera polisacharydy i skrobię oporną, które są skutecznie metabolizowane przez mikrobiotę jelitową i organizmy probiotyczne [40,41]. Fakty te mogą wskazywać, że kiełki wspomnianego pseudozboża mogą być skutecznym nośnikiem i prebiotykiem - stymulującym wzrost i przeżywalność drożdżaków probiotycznych w niskoprzetworzonej żywności oraz w przewodzie pokarmowym człowieka. Można również przypuszczać, że fortyfikacja kiełków drożdżami probiotycznymi może pozytywnie wpłynąć na wartość odżywczą oraz właściwości prozdrowotne otrzymanych kiełków gryki zwyczajnej, w zmienionych warunkach wzrostu nasion. Zgodnie z wiedzą autorki nie przeprowadzono do tej pory badań oceniających wpływ modyfikacji środowiska wzrostu nasion z zastosowanie dodatku probiotycznych drożdży *Saccharomyces cerevisiae* var. *boulardii* na wartość odżywczą i prozdrowotną kiełków gryki.

2. CEL PRACY I HIPOTEZY BADAWCZE

Zastosowanie odpowiednich warunków w trakcie kiełkowania może wpłynąć na pozyskanie surowca o zmienionych właściwościach odżywczych oraz prozdrowotnych. Stąd też głównym celem pracy była ocena wpływu modyfikacji środowiska wzrostu gryki *Fagopyrum esculentum* Moench poprzez wprowadzenie, w trakcie kiełkowania dodatku probiotycznych drożdży *Saccharomyces cerevisiae* var. *boulardii* na wartość odżywczą i prozdrowotną otrzymanego surowca.

Cele szczegółowe:

1. Zastosowanie modyfikacji środowiska wzrostu nasion gryki zwyczajnej poprzez dodatek probiotycznego szczepu drożdży oraz otrzymanie kiełków gryki w postaci liofilizatu
2. Określenie wartości odżywczej liofilizatu kiełków
3. Określenie wybranych właściwości prozdrowotnych liofilizatu kiełków w badaniach *in vitro* i *in vivo*

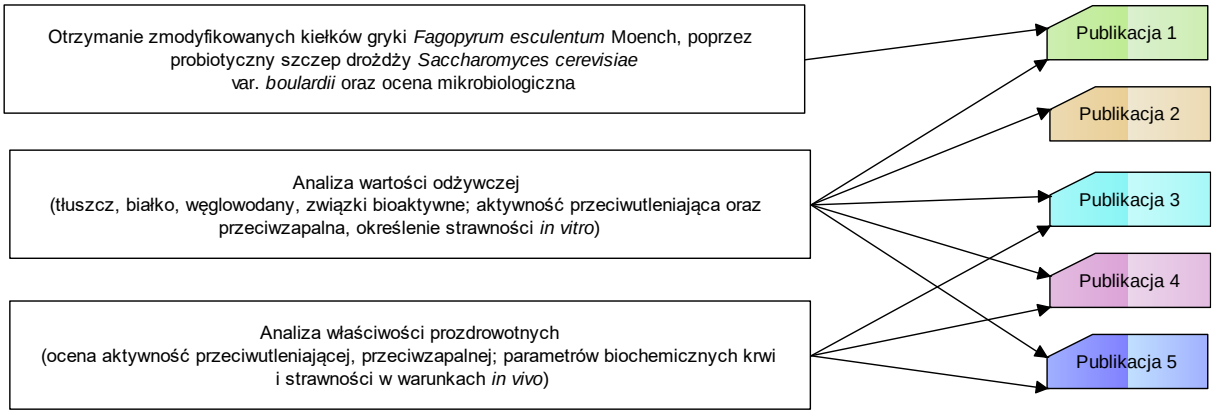
Aby zrealizować założone cele postawiono następujące hipotezy badawcze:

1. Kiełki gryki pozyskane w obecności drożdży probiotycznych różnią się istotnie zawartością oraz profilem składu podstawowego surowca, w porównaniu z kiełkami kontrolnymi
2. Biodostępność białka i skrobi uległa zmianie w kiełkach zmodyfikowanych w porównaniu z kiełkami kontrolnymi
3. Zmodyfikowane kiełki gryki różnią się istotnie zawartością i/lub profilem związków bioaktywnych surowca (związków fenolowych, flawonoidów), w porównaniu z kiełkami kontrolnymi
4. Modyfikacja środowiska wzrostu kiełków gryki poprzez zastosowanie dodatku drożdży probiotycznych wpływa na ich aktywność przeciwutleniającą i przeciwzapalną
5. Dodatek zmodyfikowanych kiełków gryki do diety aterogennej szczurów wpływa na parametry ogólnożywieniowe i biochemiczne krwi.

3. ZAKRES BADAŃ

Na rycinie 1 przedstawiono ogólny zakres badań, w którym przedstawiono również informacje odnoszące się do zawartości merytorycznej publikacji wchodzących w skład cyklu rozprawy. Otrzymano zmodyfikowane kiełki gryki *Fagopyrum esculentum* Moench, poprzez dodatek do środowiska wzrostu probiotycznego szczepu drożdży *Saccharomyces cerevisiae* var. *boulardii* oraz oceniono wartość odżywczą i prozdrowotną otrzymanego surowca.

W celu łatwiejszej interpretacji wyników, uzyskane kiełki w wyniku modyfikacji środowiska wzrostu poprzez dodatek probiotycznych drożdży nazwano jako **kiełki zmodyfikowane.**



Rycina 1. Ogólny zakres badań

PUBLIKACJA 1: Molska, M.; Reguła, J.; Rudzińska, M.; Świeca, M. Fatty Acids Profile, Atherogenic and Thrombogenic Health Lipid Indices of Lyophilized Buckwheat Sprouts Modified with the Addition of *Saccharomyces Cerevisiae* Var. *Boulardii*. *Acta Scientiarum PolonorumTechnologia Alimentaria* 2020, *19*, 483–490, http://doi.org/10.17306/ J.AFS.2020.0866.

PUBLIKACJA 2: Molska, M.; Reguła, J.; Zielińska-Dawidziak, M.; Tomczak, A.; Świeca, M. Starch and Protein Analysis in Buckwheat (*Fagopyrum Esculentum* Moench) Sprouts Enriched with Probiotic Yeast. *LWT* 2022, 113903, https://doi.org/ 10.1016/j.lwt.2022.113903.

PUBLIKACJA 3: Molska, M.; Reguła, J.; Kapusta, I.; Świeca, M. Analysis of Phenolic Compounds in Buckwheat (*Fagopyrum Esculentum* Moench) Sprouts Modified with Probiotic Yeast. *Molecules* 2022, *27*, 7773, https://doi.org/10.3390/molecules27227773.

PUBLIKACJA 4: Molska, M.; Reguła, J.; Świeca, M. Adding Modified Buckwheat Sprouts to an Atherogenic Diet - the Effect on Selected Nutritional Parameters in Rats. Plant Foods for Human Nutrition 2023, https://doi.org/10.1007/s11130-023-01047-9.

PUBLIKACJA 5: Molska, M.; Reguła, J.; Grygier, A.; Muzsik-Kazimierska, A.; Rudzińska, M.; Gramza-Michałowska, A. Effect of the Addition of Buckwheat Sprouts Modified with the Addition of *Saccharomyces Cerevisiae* Var. *Boulardii* to an Atherogenic Diet on the Metabolism of Sterols, Stanols and Fatty Acids in Rats. *Molecules* 2022, *27*, 4394, https://doi.org/ 10.3390/molecules27144394.

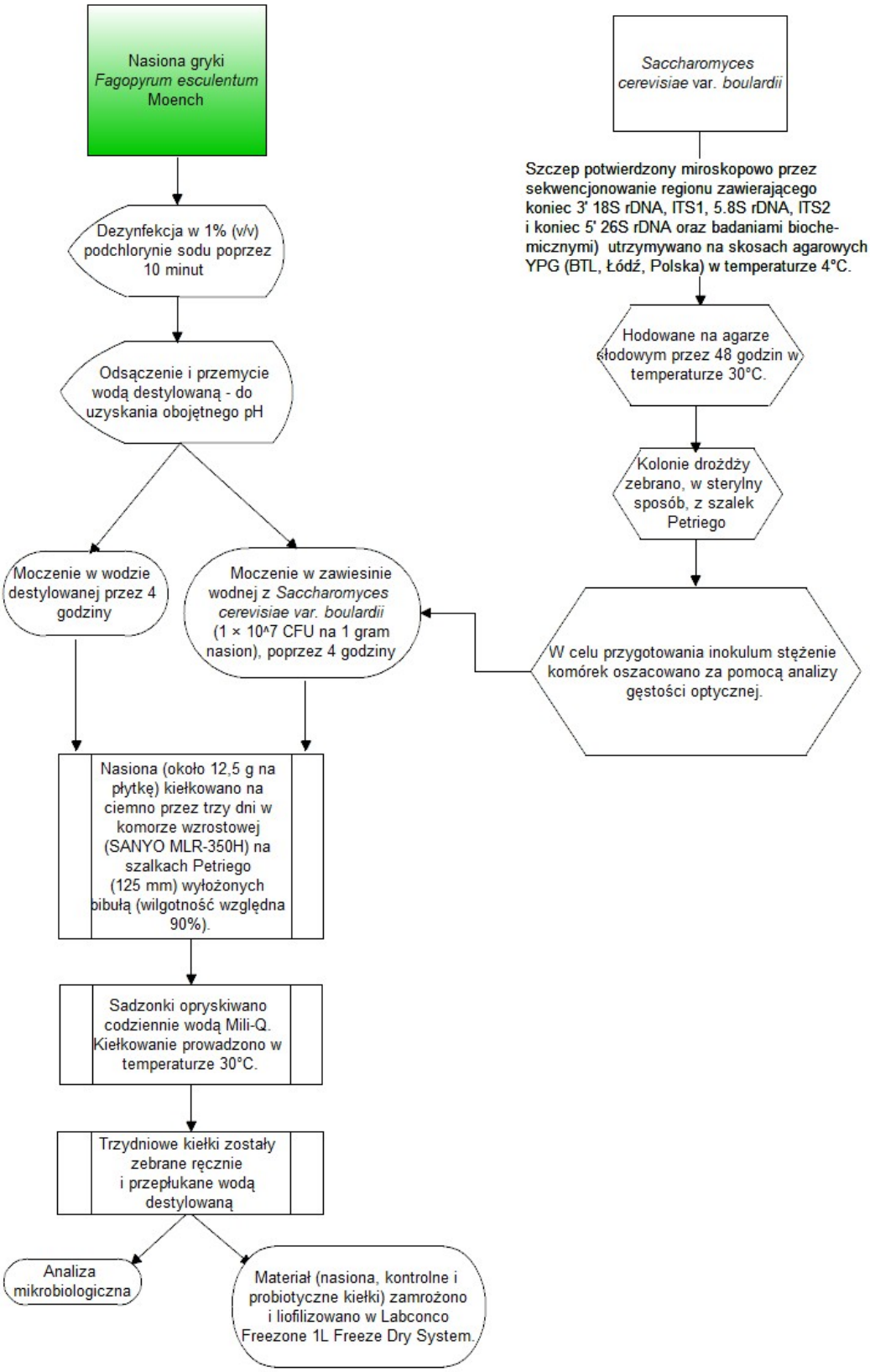
4. PRZEDMIOT BADAŃ I METODYKA BADAWCZA

4.1. Materiał badawczy

Materiał badawczy stanowiły skiełkowane nasiona gryki *Fagopyrum esculentum* Moench zmodyfikowane poprzez dodatek w trakcie wzrostu probiotycznego szczepu drożdży *Saccharomyces cerevisiae* var. *boulardii*.

- Nasiona gryki zwyczajnej *Fagopyrum esculentum* Moench pozyskano z PNOS SA, Ożarów Mazowiecki, Polska.
- Szczep *Saccharomyces cerevisiae var. boulardii* został potwierdzony mikroskopowo przez sekwencjonowanie regionu zawierającego koniec 3' 18S rDNA, ITS1, 5.8S rDNA, ITS2 i koniec 5' 26S rDNA oraz badaniami biochemicznymi.

Opis metodyki otrzymania zmodyfikowanych kiełków gryki przedstawiono na rycinie 2, jak również w publikacji 1 (wchodzącej w skład cyklu).



Rycina 2. Metodyka otrzymania zmodyfikowanych kiełków gryki *Fagopyrum esculentum* Moench, z wykorzystaniem *Saccharomyces cerevisiae* var. *boulardii*

4.2. Zastosowane metody analizy

4.2.1. Analiza mikrobiologiczna

Całkowitą zawartość mezofilnych bakterii oceniono według PN-EN ISO 4833-2:2013; natomiast drożdży i pleśni według PN-ISO 21527-1:2009 [42,43] (publikacja 1).

4.2.2. Ocena wartości odżywczej

Na rycinie 4 przedstawiono zakres przeprowadzonych badań

Analiza zawartości tłuszczu całkowitego, kwasów tłuszczowych, fitosteroli i steroli

a) Zawartość tłuszczu w nasionach, kiełkach, dietach, materiale biologicznym (według AOAC Official Method 945.16) [44] (publikacja 1, 4 i 5)
b) Analiza kwasów tłuszczowych
    - nasiona, kiełki kontrolne oraz kiełki zmodyfikowane: ekstrakcja tłuszczu według metody Folcha; estry metylowe kwasów tłuszczowych otrzymano zgodnie AOCS Official Method Ce 2-66 i AOCS Official Method Ce 2b-11, [45–47] (publikacja 1 i 5)
    - wątroba: ekstrakcja tłuszczu według zmodyfikowanej metody Folcha (publikacja 5); estry metylowe kwasów tłuszczowych otrzymano zgodnie AOCS Official Method Ce 2b-11, [45–48] (publikacja 5)
    - kał: ekstrakcja tłuszczu według metody Folcha; estry metylowe kwasów tłuszczowych otrzymano zgodnie AOCS Official Method Ce 2b-11, [45–47] (publikacja 5)
    - diety: ekstrakcja tłuszczu według metody Folcha; estry metylowe kwasów tłuszczowych otrzymano zgodnie AOCS Official Method Ce 2b-11, [45–47] (publikacja 5)
    - surowica: ekstrakcja tłuszczu według zmodyfikowanej metody Folcha, [45,49] (publikacja 5);
    - Indeksy związane z jakością tłuszczu: obliczone wg wzorów w publikacji Ulbricht and Southgate [50] oraz Sinkovič i in. [51] (publikacja 1)
c) Analiza zawartości fitosteroli/steroli
    - nasiona oraz kiełki: ekstrakcja tłuszczu według metody Folcha; zawartość steroli wg AOCS Official Method Ch 6-91,[45,52] (publikacja 5)
    - wątroba: ekstrakcja tłuszczu według zmodyfikowanej metody Folcha (publikacja 5); zawartość steroli wg AOCS Official Method Ch 6-91,[45,48,52] (publikacja 5)

- kał: ekstrakcja tłuszczu według metody Folcha; zawartość steroli wg AOCS Official Method Ch 6-91, [45,52] (publikacja 5)
- diety: ekstrakcja tłuszczu według metody Folcha; zawartość steroli wg AOCS Official Method Ch 6-91, [45,52] (publikacja 5)
- surowica: : ekstrakcja tłuszczu według zmodyfikowanej metody Folcha (publikacja 5); zawartość steroli wg AOCS Official Method Ch 6-91,[45,49,52] (publikacja 5)

Analizy związane z białkiem

- Frakcje białka (oznaczone wg zmodyfikowanej metodyki Ribeiro, Teixeira, i Ferreira [53] (publikacja 2)
- Całkowita zawartość białka według Bradford [54] (publikacja 2)
- Wolne aminokwasy i peptydy (oznaczone z wykorzystaniem metod wg Periago, Ros, Martines i Rincon [55], Lowry, Rosenbrough, Farr i Randall [56], Sun i in. [57] (publikacja 2)
- Skład aminokwasowy (oznaczone wg AOAC SMPR 2014.013 [58], tryptofan oznaczony spektrofotometrycznie wg AOAC 988.15-1988) [59] (publikacja 2)
- Trawienie białka *in vitro* (przeprowadzone wg metodyki Minekus i in. [60] z modyfikacjami Sęczyk i in. [61] (publikacja 2)
- Strawność białka na podstawie zawartości białka przed (TP; suma frakcji białkowych oznaczona zgodnie z procedurą opisaną w punkcie 5) i po trawieniu *in vitro* oceniono strawność białka (PD). W celu określenia zawartości białka po procedurze trawienia peletki frakcjonowano zgodnie z procedurą opisaną przez Chang i in. [62] (publikacja 2)

Analizy zawiązane z wolnymi cukrami, błonnikiem pokarmowym, skrobią:

- Wolne cukry (oznaczone wg Kim, Kim i Park [63] (publikacja 2)
- Błonnik pokarmowy: zawartość całkowitego błonnika pokarmowego (TDF), nierozpuszczalnego błonnika pokarmowego (IDF) i rozpuszczalnego błonnika pokarmowego (SDF) mierzono za pomocą zestawy do analizy całkowitego błonnika pokarmowego Megazyme International Ireland Ltd., Wicklow, Irlandia [64] (publikacja 3)
- Całkowita zawartość skrobi: oznaczono w wykorzystaniem metodyki Goni, Garcia-Alonso o Saura-calixio [65,66], zawartość glukozy wg Miller [65,66], całkowita zawartość skrobi obliczono jako glukozę x 0,9 (publikacja 2)

- Skrobia oporna (RS) i potencjalnie biodostępna skrobia (AS): analizowano na podstawie wyników uzyskanych po symulowanym trawieniu żołądkowo-jelitowym. Skrobię oporną (RS) obliczono jako glukozę x 0,9. Zawartość potencjalnie biodostępnej skrobi (AS) obliczono jako różnice między TS i RS., (publikacja 2)
- Całkowita zawartość wolnych cukrów: procedurę przeprowadzono zgodnie z protokołem testu Megazyme (zestaw Sacharoza/D-fruktoza/D-glukoza) i publikacją Steegmansa i in. [67,68] (publikacja 2)
- Trawienie skrobi *in vitro* (przeprowadzone wg metodyki Minekus i in. [60] z modyfikacjami Sęczyk i in. [61] (publikacja 2)
- Strawność skrobi: oceniono na podstawie zawartości skrobi całkowitej oraz skrobi opornej oznaczanej po trawieniu *in vitro,* (publikacja 2)
- Kinetyka trawienia skrobi *in vitro* i oczekiwany indeks glikemiczny (eGI): przeprowadzono zgodnie z procedurą Goni i in. wzorem Granfeldt, Bjorck, Drews i Tovar [69], ładunek glikemiczny obliczono zgodnie z Venn i in. [70] (publikacja 2)

Analiza czynników wpływających na strawność składników pokarmowych

- Ekstrakcja inhibitorów hydrolaz: w celu ekstrakcji 100 mg kiełków gryki zawieszono w 4 ml 50 mM buforu fosforanowego, pH 7,6, następnie mieszano przez 2 godziny w temperaturze 4°C i wirowano przy 9000xg przez 20 min. Supernatant użyto do dalszej analizy. (publikacja 3)
- Aktywność inhibitora trypsyny: oznaczono wg Kakade, Rackis, McGhee i Puski [71] (publikacja 3)
- Aktywność inhibitora alfa-amylazy: oznaczona wg zmodyfikowanej metody Deshpande i Cheryan [72] (publikacja 3)
- Aktywność inhibitora amyloglukozydazy: oznaczona wg zmodyfikowanej metody Anson [73] (publikacja 3)
- Całkowita zawartość fenoli (TPC): ekstrakcja wg metody opisanej w Bochniak-Niedźwiecka, Szymanowska i Świeca [74], całkowita zawartość związków fenolowych wg Singleton, Orthofer i Lamuela-Raventos [75] (publikacja 3)
- Taniny skondensowane: oznaczone wg metody opisanej w Sun, Ricardo-da-Silca i Spranger [76] (publikacja 3)

Określenie zawartości fenoli:

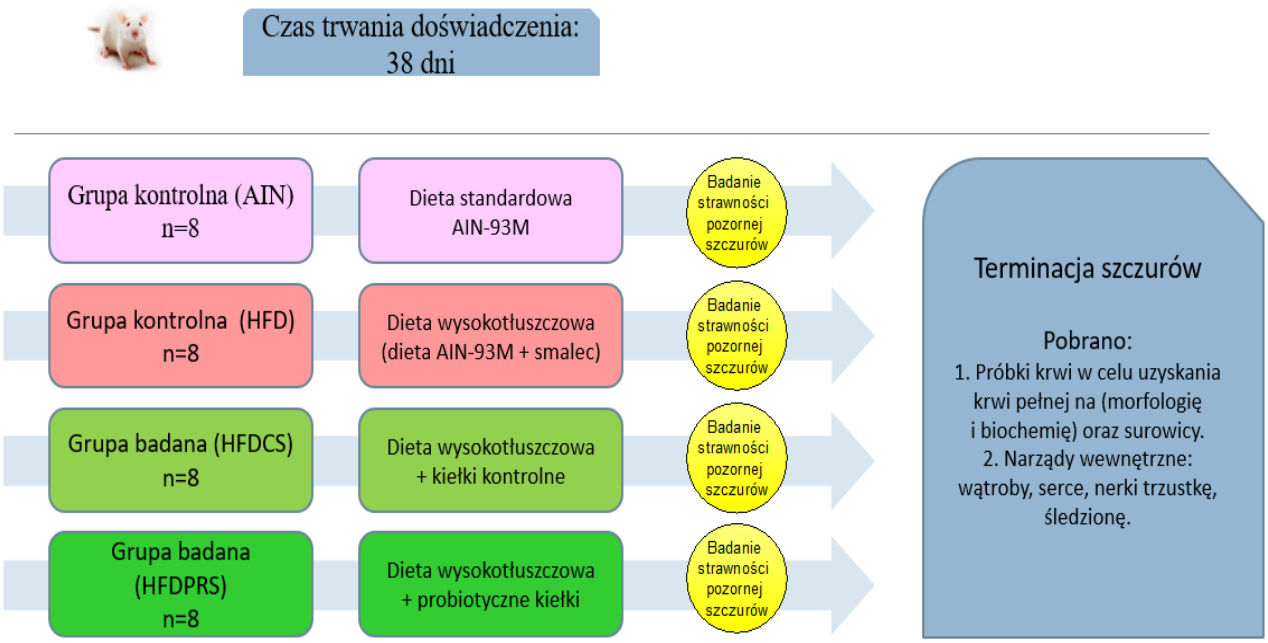
- Procedura ekstrakcji: liofilizowane próbki nasion i kiełków (100 mg) zmieszano z 5 ml 50% metanolu. Próbki poddano działaniu ultradźwięków w temperaturze pokojowej (25 ± 1◦C) (3 odstępy czasu po 30 s; 42 kHz, 135 W; Branson Ultrasonic Corporation, Brookfield, WI, USA) i ekstrahowano przez 30 minut w wytrząsarce roboczej (MS3 Basic, Ika , Wilmington, DE, USA, 150 obr./min.). Następnie próbki odwirowano (20 min 6800 x g). Otrzymane supernatanty trzymano w temperaturze -20 ◦C przed analizą jako ekstrakty chemiczne (publikacja 3)
- Jakościowo-ilościowa analiza związków fenolowych: oznaczono metodą (UPLC)-PDA-MS/MS Waters ACQUITY (Waters. Milford, MA, USA); Określenie całkowitej zawartości związków fenolowych jako suma poszczególnych związków (publikacja 3)
- Strawność *in vitro* dot. aktywności przeciwutleniającej i przeciwzaplanej oraz flawonoidów: przeprowadzone wg metodyki Minekus i in. z modyfikacjami Sęczyk i in. [61] (publikacja 3)
- Analiza ilościowa całkowitej zawartości flawonoidów (TFC): oznaczone wg Haile i Kang [77] (publikacja 3)
- Określenie wskaźnika strawności w symulowanych warunkach *in vitro* (gastrointestinal digestibility index (IA)), dot. aktywności przeciwutleniającej i przeciwzaplanej oraz flawonoidów w przewodzie pokarmowym: obliczone wg Gawlik-Dziki i in. [78] (publikacja 3)

4.2.3. Ocena wybranych właściwości prozdrowotnych

a) Zdolność do wygaszania kationorodników ABTS: oznaczenie wg Re i in. [79] (publikacja 3)
b) Siła redukcji: oznaczono wg Pulido, Bravo i Saura-Calixto [80] (publikacja 3)
c) Zdolność chelatowania metali: oznaczono wg metody Guo i in. [81] (publikacja 3)
d) Zdolność do hamowania aktywności cyklooksygenaz (COX-1 i COX-2): określono przy użyciu kolorymetrycznego testu przesiewowego inhibitora COX (Cayman Chemical, nr 701050), (publikacja 3)
e) Zdolność do hamowania aktywności lipooksygenazy (LOX): oznaczone wg metody opisanej w publikacji Szymanowskiej i in. [82] (publikacja 3)

4.3. Przebieg modelowego doświadczenia *in vivo*

Doświadczenie uzyskało pozytywną zgodę lokalnej komisji etycznej przy Uniwersytecie Medycznym w Poznaniu nr 28/2017. Na rycinie 3 przedstawiono schemat badań *in vivo.*

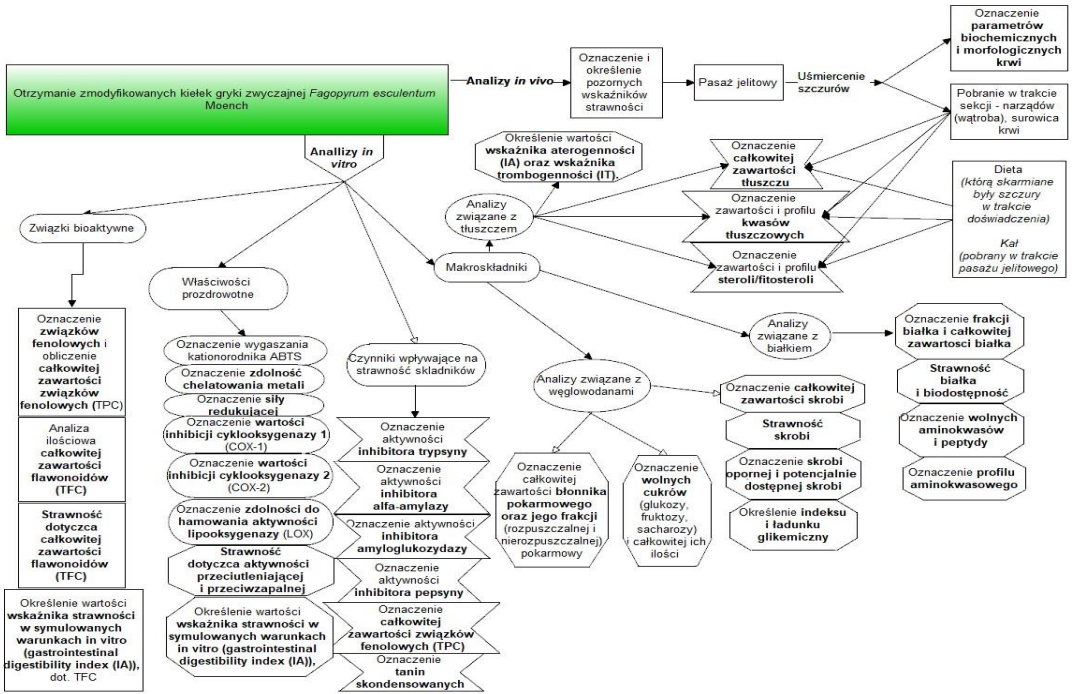


Rycina 3. Model badań na zwierzętach

4.3.1. Zwierzęta

W badaniu zostały uwzględnione 8-tygodniowe, 32 samce szczurów rasy Wistar. Na początku, w trakcie trzydniowego okresu adaptacyjnego zwierzęta miały nieograniczony dostęp do standardowej diety AIN-93M oraz wody [83]. Przez cały czas eksperymentu szczury były trzymane w klatkach ze stali nierdzewnej pokrytych szkliwem niemetalowym.

Zwierzęta zostały podzielono losowo na cztery grupy (Ryc. 3). Jedna z grup była karmiona zmodyfikowaną kontrolną dietą AIN-93M; kolejne trzy grupy żywiono zmodyfikowaną dietą AIN-93M z dodatkiem smalcu w ilości 200 g/kg dla diety HFD i 200 g/1,3 kg dla diet HFDCS i HFDPRS (Tabela 2) [83]. W dietach HFDCS i HFDPRS uwzględniono dodatek odpowiednio 600 g liofilizatu z kiełków kontrolnych i kiełków zmodyfikowanych. Codziennie kontrolowano spożycie diet oraz wody, a raz w tygodniu mierzono masę ciała. Pod koniec eksperymentu, szczury zostały zważone. Zwierzęta zostały uśmiercone za pomocą inhalacji dwutlenkiem węgla. Do dalszych analiz pobrano krew i narządy.



Rycina 4. Zestawienie badań wykonanych w ramach pracy doktorskiej

.

Tabela 2. Kompozycja diet eksperymentalnych (g/kg – AIN-93M, HFD; g/1,3kg – HFDCS, HFDPRS)

Składnik	AIN-93M g/kg	HFD[1] g/kg	HFDCS[2] g/kg	HFDPRS[3] g/kg
Skrobia pszenna	627,5	422,5	422,5	422,5
Skrobia ziemniaczana	50	50	50	50
Smalec	-	200	200	200
Sacharoza	100	100	100	100
Mieszanka mineralna [83]	35	35	35	35
Mieszaka witaminowa [83]	10	10	10	10
Cholina	2,5	2,5	2,5	2,5
Kiełki kontrolne	-	-	600	-
Zmodyfikowane kiełki gryki	-	-	-	600

[1] HFD – AIN-93M+ smalec (dieta wysokotłuszczowa) [2]HFDCS – dieta wysokotłuszczowa + kiełki kontrolne [3]HFDPRS – dieta wysokotłuszczowa + zmodyfikowane kiełki

4.3.2. Określenie strawności pozornej

Parametry dotyczące strawności określano w trakcie 10 dni. W tym okresie, zbierano kał, który suszono od razu po pobraniu, poprzez 24 godziny, w temperaturze 60°C.

Stosując standardowe metody analityczne oznaczono, suchą masę, białko oraz tłuszcz surowy w zebranym kale oraz diecie. Suchą masę określono przez suszenie 1g każdej próbki, w piecu, poprzez 12 godzin, w temperaturze 105°C i ważenie. Białko oznaczono metodą Kjeldahla, zawartość tłuszczu całkowitego oznaczono metodą ekstrakcji Soxhleta, natomiast, węglowodany obliczono jako 100 minus różnicę wszystkich pozostałych składników czyli wody, białka, tłuszczy i popiołu [84].

Pozorny współczynnik strawności składników pokarmowych diet obliczono: Pozorna strawność = 100 × (NI − NE)/NI gdzie NI oznacza pobranie składników odżywczych, a NE – wydalanie składników pokarmowych.

W 11 dniu doświadczenia określono również czas przejścia treści pokarmowej przez przewód pokarmowy zwierząt metodą wskaźnikową, stosując 1 g tlenku chromu(III)/100 g diety w ciągu 24 godzin, według metody opracowanej przez Gobla i Gohla [85]. Za pomocą ekwiwalentów Atwatera obliczono wartość energetyczną diety [86], a na podstawie skumulowanego spożycia diety i przyrostów masy ciała wyliczono wskaźnik wykorzystania paszy [87].

4.3.3. Analiza parametrów hematologicznych i biochemicznych

We krwi oznaczono następujące parametry morfologiczne: krwinki białe (WBC), średnia objętość krwinek czerwonych (MCV), średnie stężenie hemoglobiny we krwi (MCH), średnia hemoglobina we krwi (MCHC), limfocyty (LYM), neutrofile (NEU), erytrocyty (RBC), monocyty (MONO), trombocyty (PLT), eozynofile (EOS), bazofile (BASO), hemoglobina (HGB), hematokryt (HCT). Badania wykonano za pomocą analizatora hematologicznego Sysmex K-1000 (TAO Medical Electronics Co., Kobe, Japonia). Oznaczono również parametry biochemiczne: glukozę (GLU), triacyloglicerole (TAG), aktywność transaminazy alaninowej (ALA), aktywność transaminazy asparaginianowej (AST), cholesterol całkowity (TCH), cholesterol lipoproteinowy o dużej gęstości (HDL), cholesterol nie-HDL i białko C-reaktywne (CRP). Stężenie glukozy w surowicy oceniano metodą oksydazy glukozowej. Poziomy cholesterolu całkowitego i triglicerydów w surowicy mierzono za pomocą komercyjnych zestawów (Randox Laboratory Ltd., Crumlin, Wielka Brytania). Aktywność enzymów wątrobowych, takich jak ALT i AST, oznaczono według Dembińskiej-Kiec i Nastalskiego [88].

Szczegółowy opis poszczególnych metodyk został uwzględniony w publikacjach stanowiących osiągnięcie naukowe.

5. OMÓWIENIE NAJWAŻNIEJSZYCH WYNIKÓW

5.1. Pozyskanie liofilizatu kiełków gryki zwyczajnej - kontrolnych i zmodyfikowanych oraz określenie wartości odżywczej i właściwości prozdrowotnych kiełków i nasion gryki – badania *in vitro*.

W uzyskanych kiełkach nasion gryki zwyczajnej przeprowadzono analizę mikrobiologiczną. Po trzech dniach kiełkowania, zauważono różnice w zawartości bakterii mezofilnych oraz drożdży pomiędzy kiełkami kontrolnymi, a zmodyfikowanymi. Wykazano, że w pojedynczej jadalnej porcji kiełków gryki zwyczajnej zmodyfikowanych poprzez drożdże probiotyczne była ilość, która klasyfikuje je jako produkt probiotyczny (6,5 log/100 g świeżej masy).

W pierwszym etapie badań określono zawartość tłuszczu całkowitego oraz procentową ilość kwasów tłuszczowych w nasionach, kiełkach kontrolnych oraz zmodyfikowanych kiełkach gryki zwyczajnej. Odnotowano wzrost ilości tłuszczu całkowitego w kiełkach (kontrolne 2,42% oraz kiełki modyfikowane 2,41%), w porównaniu z nasionami (1,62%). W nasionach gryki zwyczajnej zidentyfikowano 13, a w kiełkach gryki zwyczajnej 11 kwasów tłuszczowych. W przypadku kwasów tłuszczowych nasyconych najwyższą ich ilość uzyskano w nasionach (21,2%). Natomiast w kiełkach ich wartość uległa zmniejszeniu. Odmienny wynik otrzymano w przypadku kwasów tłuszczowych nienasyconych, gdzie zauważono zwiększenie ilości kwasów nienasyconych (nasiona 78,48%, kiełki kontrolne 83,01%, zmodyfikowane kiełki 83,16%). Wykazano niższą ilość następujących kwasów w kiełkach zmodyfikowanych, w porównaniu z kiełkami kontrolnymi: kwas plamitynowy, kwas ginkgolowy, kwas oleinowy. Z kolei większą ilość zanotowano kwasu arachidonowego, kwasu eikozenowego i kwasu linolenowego.

Na podstawie danych dotyczących składu kwasów tłuszczowych obliczono wskaźnik aterogenności (IA) oraz wskaźnik trombogenności (IT). Kiełki kontrolne oraz zmodyfikowane odznaczały się niższą wartością powyższych wskaźników w porównaniu z nasionami. Wskaźnik aterogenności stanowił odpowiednio 0,22 (nasiona) oraz 0,17 (kiełki kontrolne oraz zmodyfikowane). Natomiast wskaźnik trombogeniczności 0,44 (nasiona), 0,32 (kiełki kontrolne i zmodyfikowane).

Powyższe - wyniki opisane na podstawie - publikacji 1:

Molska, M.; Reguła, J.; Rudzińska, M.; Świeca, M. Fatty Acids Profile, Atherogenic and Thrombogenic Health Lipid Indices of Lyophilized Buckwheat Sprouts Modified with the Addition of *Saccharomyces Cerevisiae* var. *Boulardii*. Acta Scientiarum Polonorum Technologia Alimentaria 2020, 19, 483–490, https://doi.org/10.17306/J.AFS.2020.0866

W drugim etapie badań, określono ilość białka oraz biodostępność - białka i skrobi w zmodyfikowanych kiełkach gryki zwyczajnej. Białko gryki składało się z 4 frakcji tj. albuminy, globuliny, prolaminy oraz gluteiny. W odniesieniu do całkowitej zawartości białka, będącej sumą poszczególnych frakcji, zmodyfikowane kiełki odznaczały się najwyższą jego ilością tj. 144 mg/g s.m., następnie kiełki kontrolne 116 mg/g s.m., nasiona 119 mg/g s.m.

W pracy podjęto próbę oceny strawności całkowitego białka i wykazano, że strawność w zmodyfikowanych kiełkach była niższa, w porównaniu z kiełkami kontrolnymi i nasionami. Parametry, które zostały zbadane to: aktywność inhibitora trypsyny, pepsyny, jak również taniny skondensowane, całkowita zawartość fenoli (TPC). Wobec, aktywności inhibitora trypsyny i pepsyny zauważono, że kiełki zmodyfikowane odznaczały się niższą aktywnością aniżeli kiełki kontrolne, jak również były pozytywnie skorelowane ze strawnością białka *in vitro*. Najwyższą ilością skondensowanych tanin oraz całkowitej zawartość fenoli odznaczały się kiełki zmodyfikowane, następnie odpowiednio kiełki kontrolne i nasiona. Były one pozytywnie skorelowane ze strawnością białka (analiza PCA).

W kolejnym etapie, określono ilości wolnych aminokwasów i peptydów, jak również profilu aminokwasów w zmodyfikowanych kiełkach gryki zwyczajnej. Najwyższą ilością wolnych aminokwasów i peptydów, podobnie, jak w przypadku całkowitego białka, odznaczały się kiełki zmodyfikowane gryki zwyczajnej, a najniższą nasiona. Kiełki zmodyfikowane odznaczały się większą, w stosunku do kiełków kontrolnych, zawartością kwasu asparaginowego, kwasu glutaminowego, tyrozyny oraz aminokwasów siarkowych.

W następnej kolejności, określono ilość oraz biodostępność skrobi w nasionach i kiełkach gryki zwyczajnej. Największą zawartością skrobi odznaczały się nasiona gryki, podczas gdy w trakcie procesu kiełkowania jej ilość uległa zmniejszeniu. Otrzymane wyniki analizy dotyczące dostępnej skrobi, również wykazały, że największą jej ilością odznaczały się nasiona, a w procesie kiełkowania ilość uległa zmniejszeniu. Ilość skrobi opornej w nasionach była największa, natomiast ilość w kiełkach zmodyfikowanych (93,69mg/g świeżej masy) była większa niż w kiełkach kontrolnych (87,83 mg/g świeżej masy).

Najmniejszą strawność skrobi stwierdzono w kiełkach zmodyfikowanych, a największą w nasionach. Strawność skrobi była pozytywnie skorelowana z aktywnością alfa-amylazy oraz amyloglukozydazy, a negatywnie skorelowana z skondensowanymi taninami i całkowitą zawartością fenoli (analiza PCA).

Powyższe - wyniki opisane na podstawie - publikacji 2:

Molska, M.; Reguła, J.; Zielińska-Dawidziak, M.; Tomczak, A.; Świeca, M. Starch and Protein Analysis in Buckwheat (Fagopyrum Esculentum Moench) Sprouts Enriched with Probiotic Yeast. LWT **2022**, 113903, https://doi.org/10.1016/j.lwt.2022.113903.

W trzecim etapie badań, przeprowadzono analizy związane z ilością związków bioaktywnych, aktywnością przeciwutleniająca i przeciwzapalną surowców oraz określono ilość oraz frakcje błonnika pokarmowego.

W kontrolnych oraz zmodyfikowanych kiełkach gryki zidentyfikowano trzy kwasy fenolowe, siedemnaście flawan-3-oli oraz cztery flawanole. Natomiast w nasionach zostały zidentyfikowane trzy kwasy fenolowe, siedemnaście flawan-3-oli oraz dwa flawanole. Sumaryczna zawartość związków fenolowych była największa w kiełkach zmodyfikowanych (1526,34 μg/g s.m.), następnie w kiełkach kontrolnych (951,42 μg/g s.m.) i nasionach (672,14 μg/g s.m.).

Podobnie w przypadku całkowitej zawartości flawonoidów, zmodyfikowane kiełki gryki odznaczały się największą ich zawartością, zarówno przed trawieniem (52,98 mg QE/g s.m.), jak i po trawieniu (15,75 mg QE/g s.m.). Odpowiednio, w kontrolnych kiełkach wartość przed trawieniem była niższa o 60,6%, a po trawieniu 50,8%.

Analiza składowych głównych (PCA) wykazała, że zarówno całkowita zawartość fenoli i flawonoidów, jak również parametry powiązane z aktywnością przeciwutleniającą i antyzapalną są ze sobą skorelowane dodatnio.

W celu analizy potencjału przeciwutleniającego i przeciwzapalnego zmodyfikowanych kiełek gryki wykorzystano następujące testy aktywności

- przeciwutleniającej:
    1. Zdolność do wygaszania kationorodników $ABTS^{+\bullet}$
    2. Zdolność chelatowania metali
    3. Siła redukująca
- przeciwzapalnej
    1. Cyklooksygenaza 1 (COX-1)
    2. Cyklooksygenaza 2 (COX-2)
    3. Lipooksygenaza (LOX)

Mając na uwadze aktywność przeciwutleniającą, wykonano w pierwszej kolejności, test wygaszania kationorodnika ABTS. Największą zdolność antyoksydacyjną względem kationorodnika ABTS wykazywały zmodyfikowane kiełki gryki, zarówno przed, jak i po trawieniu (19,78 mg TE/1 g; 11,05 mg TE/1 g). Analiza zdolności chelatowania metali pokazała, że przed trawieniem nie odnotowano różnic istotnych statystycznie pomiędzy kiełkami kontrolnymi, a zmodyfikowanymi. Jednakże sytuacja wyglądała inaczej w przypadku biodostępności, bowiem w tym przypadku zauważono największą jej wartość w przypadku

kiełków zmodyfikowanych. Analogiczne wyniki, jak w przypadku wygaszania kationorodnika ABTS, otrzymano dla siły redukującej. Wartości otrzymane przed trawieniem *in vitro* kiełki zmodyfikowane (31,15 mg TE/g s.m.), kiełki kontrolne (17,59 mg TE/g s.m.), nasiona (11,24 mg TE/g s.m.). Natomiast po trawieniu kiełki zmodyfikowane (11,24 mg TE/g s.m.), kiełki kontrolne (6,50 mg TE/g s.m.), nasiona (2,87 mg TE/g s.m.).

W związku z aktywnością przeciwzapalną oznaczono wartość inhibicji cyklooksygenazy 1 i 2 oraz lipooksygenazy. Oznaczenia zostały wykonane przed oraz po trawieniu *in vitro*. Odnotowano, że zarówno w przypadku COX-1, COX-2 oraz LOX największą wartością inhibicji odznaczały się kiełki zmodyfikowane, odpowiednio przed trawieniem 3037,15 IU/g s.m., 2421,24 IU/g s.m., 4721,56 IU/g s.m.; po trawieniu 904,00 IU/g s.m., 470,00 IU/g s.m, 1250,00 IU/g s.m. W przypadku biodostępności zauważono, że największą jej wartością w odniesieniu do COX-1 odznaczały się kiełki kontrolne, w przypadku COX-2 kiełki zmodyfikowane, a w kwestii LOX nasiona.

W kolejnym etapie, określono ilość oraz frakcje błonnika pokarmowego w kiełkach gryki oraz nasionach. Zważywszy na poszczególne frakcje błonnika pokarmowego, kiełki zmodyfikowane zawierały największą ilość frakcji rozpuszczalnej, a tym samym całkowitej frakcji błonnika (16,11%) w porównaniu z kiełkami kontrolnymi i nasionami. Analiza składowych głównych (PCA) wykazała, że zarówno rozpuszczalny błonnik pokarmowy, jak i całkowita ilość błonnika były dodatnio skorelowane z całkowitą ilością fenoli i flawonoidów, jak również z parametrami związanymi z aktywnością przeciwutleniającą i antyzapalną.

Powyższe - wyniki opisane na podstawie - publikacji 3:
Molska, M.; Reguła, J.; Kapusta, I.; Świeca, M. Analysis of Phenolic Compounds in Buckwheat (Fagopyrum Esculentum Moench) Sprouts Modified with Probiotic Yeast. Molecules 2022, 27, 7773, https://doi.org/10.3390/molecules27227773.

5.2. Analiza parametrów ogólnożywieniowych i prozdrowotnych u szczurów spożywających diety aterogenne z dodatkiem kiełków gryki

<u>W pierwszym etapie badań</u> oceniono parametry ogólnożywieniowe i morfologiczne krwi szczurów.

W trakcie doświadczenia modelowego *in vivo* szczury karmiono czterema dietami: dieta AIN-93M (dieta podstawowa), HFD (dieta podstawowa AIN-93M, z dodatkiem smalcu – dieta wysokotłuszczowa), HFDCS (dieta wysokotłuszczowa, z dodatkiem kiełków kontrolnych),

HFDPRS (dieta wysokotłuszczowa z dodatkiem zmodyfikowanych kiełków). Dieta HFDPRS odznaczała się w porównaniu z dietą HFDCS większą ilością białka oraz tłuszczu.

Badanie parametrów ogólnożywieniowych nie wykazało różnic istotnych statystycznie pomiędzy grupą karmioną dietą aterogenną z dodatkiem kiełków kontrolnych (HFDCS), a grupą żywioną dietą aterogenną z dodatkiem kiełków zmodyfikowanych (HFDPRS). Nie stwierdzono również istotnych różnic w parametrach morfologicznych krwi we wszystkich grupach.

Powyższe - wyniki opisane na podstawie - publikacji 4:
Molska, M.; Reguła, J.; Świeca, M. Adding Modified Buckwheat Sprouts to an Atherogenic Diet – the Effect on Selected Nutritional Parameters in Rats. Plant Foods for Human Nutrition 2023, https://doi.org/10.1007/s11130-023-01047-9.

W kolejnych badaniach przeprowadzono ocenę parametrów biochemicznych szczurów oraz analizę zawartości steroli/fitosteroli, kwasów tłuszczowych w materiale biologicznym, dietach, nasionach i kiełkach.

W trakcie modelowego doświadczenia *in vivo* zostały wykonane badania biochemiczne krwi, w których nie zaobserwowano różnic istotnych statystycznie pomiędzy szczurami karmionymi dietą aterogenną z dodatkiem liofilizatu kiełków kontrolnych i zmodyfikowanych. W materiale biologicznym uzyskanym od zwierząt (surowica krwi, wątroba, kał), dietach, nasionach oraz kiełkach kontrolnych i zmodyfikowanych - oznaczono tłuszcz całkowity, fitosterole/sterole, kwasy tłuszczowe.

Tłuszcz całkowity

Zaobserwowano, że najwyższą ilością tłuszczu odznaczała się dieta HFD (23,91 g/100g próbki), w dalszej kolejności odpowiednio dieta HFDCS (19,07 g/100g próbki) i HFDPRS (19,34 g/100g próbki), AIN-93M (4,41 g/100g próbki).

Grupa zwierząt żywiona dietą AIN-93M odznaczała się najmniejszą ilością tłuszczu w wątrobie (4,44 g/100g próbki), następnie grupa HFDPRS (4,74 g/100g próbki), HFD (4,99 g/100g), HFDCS (5,14 g/100g próbki).

W odniesieniu do zawartość tłuszczu w kale - odnotowano, że również grupa żywiona dietą AIN-93M cechowała się najmniejszą zawartością tłuszczu, spośród badanych grup (1,65g/100g próbki). Natomiast najwyższą statystycznie istotną wartością odznaczała się grupy żywione dietami HFDCS (3,25 g/100g próbki) i HFDPRS (3,36 g/100g próbki).

Sterole/Fitosterole

Nie stwierdzono istotnych różnic pomiędzy nasionami, kontrolnymi, a zmodyfikowanymi kiełkami, w kontekście zawartość w nich fitosteroli. W przypadku diet najmniejszą ilość fitosteroli odnotowano w diecie HFD, potem HFDCS>HFDPRS>AIN.

W surowicy zidentyfikowano sterole: cholesterol oraz sitostanol. Przy czym należy zaznaczyć, że sitostanol nie został zidentyfikowany w surowicy szczurów karmionych dietą HFD. W wątrobach zauważono istotne różnice pomiędzy grupą HFDPRS, a HFDCS i HFD. Nie zidentyfikowano w wątrobach szczurów w grupach HFDPRS oraz HFD sitostanolu.

Kał odznaczał się zmianą profilu steroli w czterech badanych próbach. W konsekwencji całkowita zawartość steroli przedstawiała się następująco grupa karmiona dietą AIN-93M (38,96 mg/g tłuszczu) i HFPRS 43,90 mg/g tłuszczu), HFDCS (62,27 mg/g tłuszczu) i HFD (72,72 mg/g tłuszczu).

Kwasy tłuszczowe

Analiza ilościowa kwasów tłuszczowych wykazała różnice w profilu i ilości kwasów tłuszczowych pomiędzy badanymi grupami. W surowcu porównując kiełki kontrolne oraz zmodyfikowane, zauważa się większą ilość nasyconych kwasów tłuszczowych, kwasów monoenowych oraz polienowych w kiełkach zmodyfikowanych. Odnosząc się do zawartości kwasów tłuszczowych w dietach aterogennych z dodatkiem kiełków, odnotowano mniejszą ilość kwasów tłuszczowych nasyconych oraz większą zawartość kwasów monoenowych w diecie HFDPRS, w porównaniu z HFDCS. W surowicy krwi szczurów zaobserwowano większą zawartość nasyconych, monoenowych oraz polienowych kwasów tłuszczowych w grupie HFDPRS. Nie stwierdzono istotnych różnic w wątrobie, aczkolwiek, zaobserwowano mniejszą ilość wydalanych kwasów tłuszczowych monoenowych oraz polienowych poprzez szczury w grupie HFDPRS, a HFDCS.

Powyższe - wyniki opisane na podstawie - publikacji 5:

Molska, M.; Reguła, J.; Grygier, A.; Muzsik-Kazimierska, A.; Rudzińska, M.; Gramza-Michałowska, A. Effect of the Addition of Buckwheat Sprouts Modified with the Addition of *Saccharomyces Cerevisiae* var. *Boulardii* to an Atherogenic Diet on the Metabolism of Sterols, Stanols and Fatty Acids in Rats. Molecules 2022, 27, 4394, https://doi.org/10.3390/molecules27144394.

6. WNIOSKI

W przeprowadzonych badaniach potwierdzono założone hipotezy badawcze:

1. Kiełki gryki pozyskane w obecności drożdży probiotycznych różnią się istotnie zawartością i/ lub profilem składu podstawowego surowca, w porównaniu z kiełkami kontrolnymi
2. Biodostępność białka i skrobi uległa zmianie w kiełkach zmodyfikowanych w porównaniu z kiełkami kontrolnymi
3. Zmodyfikowane kiełki gryki różnią się istotnie zawartością i/ lub profilem związków bioaktywnych surowca (związków fenolowych, flawonoidów), w porównaniu z kiełkami kontrolnymi
4. Modyfikacja środowiska wzrostu kiełków gryki poprzez zastosowanie dodatku drożdży probiotycznych wpływa na ich aktywność przeciwutleniającą i przeciwzapalną

Badanie *in vitro*:

1. Ilość tłuszczu całkowitego w kiełkach była większa w porównaniu do nasion. W kiełkach zmodyfikowanych zanotowano niższą ilość kwasu palmitynowego, kwasu ginkgolowego, kwasu oleinowego, a większą kwasu arachidonowego, kwasu eikozenowego i kwasu linolenowego w porównaniu z kiełkami kontrolnymi.
2. Nie odnotowano różnic w ilości i profilu fitosteroli w kiełkach kontrolnych i zmodyfikowanych.
3. Większa zawartość białka całkowitego i skrobi opornej była w kiełkach zmodyfikowanych, w porównaniu z kontrolnymi. Biodostępność białka z kiełków zmodyfikowanych była niższa w porównaniu z pozostałymi badanymi próbami.
4. Odnotowano większą ilość wolnych aminokwasów i peptydów w zmodyfikowanych kiełkach, w porównaniu z grupami kontrolnymi, jak również zmiany ilości aminokwasów pomiędzy badanymi grupami. Większą zawartość aminokwasów siarkowych stwierdzono w zmodyfikowanych kiełkach gryki.
5. Zmodyfikowane kiełki gryki odznaczały się największą całkowitą zawartością związków fenolowych. Większa ilość związków fenolowych w zmodyfikowanych kiełkach gryki, związana była z większą ich aktywnością antyoksydacyjną, w porównaniu z kiełkami kontrolnymi.
6. Zmodyfikowane kiełki gryki odznaczały się większą aktywnością przeciwutleniającą i przeciwzapalną, w porównaniu z kiełkami kontrolnymi.

Badanie *in vivo*:

1. Odnotowano istotne różnice w ilości makroskładników w diecie z dodatkiem liofilizatu zmodyfikowanych kiełków gryki, w porównaniu z dietą z kontrolnymi kiełkami.
2. Dodatek zmodyfikowanych kiełków gryki do diety aterogennej szczurów nie wpłynął na parametry ogólnożywieniowe i biochemiczne krwi
3. Zauważono wpływ dodatku do diety wysokotłuszczowej zmodyfikowanych kiełków gryki na poziom steroli oraz kwasów tłuszczowych w organizmie szczura.

Zmodyfikowane kiełki gryki stanowią potencjał pod kątem możliwości i zakresu dalszych badań. Kiełki gryki zwyczajnej zmodyfikowane poprzez *S. cerevisiae* var. *boulardii* to nowy produkt, który charakteryzuje się pożądaną wartością odżywczą i prozdrowotną, posiadający wysoką zawartość związków bioaktywnych, wysoki potencjał antyoksydacyjny i przeciwzapalny. Może w związku z powyższym zostać wykorzystany jako potencjalny dodatek do produktów spożywczych, szczególnie dla osób cierpiących z powodu przewlekłych chorób niezakaźnych.

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9. SPIS RYCIN I TABEL

Tabele

Ryciny

10.OŚWIADCZENIA WSPÓŁAUTORÓW ORAZ PUBLIKACJE STANOWIĄCE PRZEDMIOT ROZPRAWY DOKTORSKIEJ

Poznań, 22.05.2023r.

Katedra Żywienia Człowieka i Dietetyki
Wydział Nauk o Żywności i Żywieniu
Uniwersytet Przyrodniczy w Poznaniu

OŚWIADCZENIE

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prof. dr hab. Magdalena Rudzińska	Katedra Technologii Żywności Pochodzenia Roślinnego, Uniwersytet Przyrodniczy w Poznaniu	MRudzińska
prof. dr hab. Michał Świeca	Katedra Biochemii i Chemii Żywności, Uniwersytet Przyrodniczy w Lublinie	Signed by / Podpisano przez: Michał Świeca Uniwersytet Przyrodniczy w Lublinie Date / Data: 2023-05-19 07:40

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dr Aneta Tomczak	Katedra Biochemii i Analizy Żywności, Uniwersytet Przyrodniczy w Poznaniu	Signed by / Podpisano przez: Aneta Tomczak Date / Data: 2023-06-02 13:44
prof. dr hab. Michał Świeca	Katedra Biochemii i Chemii Żywności, Uniwersytet Przyrodniczy w Lublinie	Signed by / Podpisano przez: Michał Świeca Uniwersytet Przyrodniczy w Lublinie Date / Data: 2023-05-19 07:40

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prof. UR dr hab. Ireneusz Kapusta	Instytut Technologii Żywności i Żywienia, Uniwersytet Rzeszowski	Ireneusz Kapusta Elektronicznie podpisany przez Ireneusz Kapusta Data: 2023.05.18 12:35:31 +02'00'
prof. dr hab. Michał Świeca	Katedra Biochemii i Chemii Żywności, Uniwersytet Przyrodniczy w Lublinie	Signed by / Podpisano przez: Michał Świeca Uniwersytet Przyrodniczy w Lublinie Date / Data: 2023-05-19 07:41

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FATTY ACID PROFILE, ATHEROGENIC AND THROMBOGENIC HEALTH LIPID INDICES OF LYOPHILIZED BUCKWHEAT SPROUTS MODIFIED WITH THE ADDITION OF *SACCHAROMYCES CEREVISIAE* VAR. *BOULARDII*

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ABSTRACT

Background. The study aims to present an assessment of the effect on the composition of fatty acids of a modification of buckwheat sprouts *Fagopyrum esculentum* Moench by the addition of the probiotic strain of yeast *Saccharomyces cerevisiae* var. *boulardii.* The study is innovative.
Materials and methods. Seeds, control and modified buckwheat sprouts lyophilisates constituted the research material. Fat analyses were performed using the standards methods. However, the determination of fatty acids was carried out following the AOCS Ce 2-66 methodology.
Results. The results indicated that the germination process increased the total fat content of the sprouts as well as changed the fatty acid profile. Statistically significant differences were found in the content of palmitic, arachidic, ginkgolic, oleic, eicosenoic and linoleic acids between the control and probiotic-rich sprouts. It was also found that the quality indicators of buckwheat lipids, such as atherogenic and thrombogenic, are optimal in terms of nutritional value.
Conclusions. Buckwheat sprouts modified by adding probiotic yeast might be a new functional product that can be used as part of a diet that reduces the risk of cardiovascular disease.

Keywords: *Fagopyrum esculentum* Moench, fatty acids, lipid indices, functional product

INTRODUCTION

Buckwheat belongs to the knotweed family, i.e. *Polygonaceae*, and is classified as a pseudocereal (Zhang et al., 2012). One of the most commonly grown species of buckwheat is *Fagopyrum esculentum* Moench. The global production of buckwheat in 2017 was 3,972,882 million tons, double the level in 2014 (1,915,966 million tonnes) (FAOSTAT, n.d.). This pseudocereal is a well-known source of health-promoting ingredients such as proteins with a high biological value, a well-balanced amino acid pattern and antioxidant

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compounds (rutin, quercetin, orientin, isoorientin, vitexin, isovitexin and vitamins). Germination has a positive effect on the biochemical changes and the nutritional value of germinated seeds (raw material). During this process, many changes occur that affect the increase or decrease in compounds contained in the raw material, including the use of compounds necessary for growth. Storage substances are broken down and also used for plant growth, which makes them easier to absorb. Fats, protein and starch are broken down into energy source compounds and substrates from which newly synthesized compounds are formed. This is essential information in the context of functional foods and industry that uses this raw material (Benincasa et al., 2019; Brajdes and Vizireanu, 2012; Lewicki, 2010; Zhang et al., 2012; Zhang et al., 2015).

Compounds contained in buckwheat exhibit a number of health-promoting properties and activities, including lowering plasma cholesterol (e.g. through buckwheat protein, flavonoids), neuroprotective effects (through tyrosinase, acetylcholinesterase, butyrylcholinesterase inhibitors, antioxidant activity; Đurendić-Brenesel et al., 2013; Gulpinar et al., 2012; Zhang et al., 2012; Zhou et al., 2015).

Probiotic microorganisms may exhibit pro-health activity through certain mechanisms of action, such as trophic effects, immune regulation and related antimicrobial properties. *Saccharomyces cerevisiae* var. *boulardii* has a wide medical application, related among other things to the prevention of antibiotic-related diarrhea and traveler's diarrhea; it also appears to be effective in preventing relapse in patients with Crohn's disease (Kelesidis and Pothoulakis, 2011; Lazo-Vélez et al., 2018; Łukaszewicz, 2012; Syngai et al., 2016). The publication by Ryan et al. (2015) indicated that supplementation with *S. boulardii* reduced the level of remnant lipoprotein, which is considered a potentially therapeutic factor in the treatment and prevention of coronary artery disease. One of the mechanisms that alters cholesterol levels is its assimilation to yeast cells (Ryan et al., 2015). It is very important to mention that this species of yeast is recommended as a biological agent that can be intentionally added to food or feed, according to the EFSA notification 8 (Ricci et al., 2018).

According to the WHO definition: "(...) when administered in adequate amounts, probiotics confer a health benefit on the host" and can be found in many products on the market, e.g. fermented dairy products, which are eliminated by people on a vegan diet (FAO and WHO, 2002; Pala et al., 2011; Tripathi and Giri, 2014). In turn, this creates a niche on the market for products that will be able to provide probiotic microorganisms to this group of consumers. In the study by Świeca et al., the possibility of using legume sprouts as carriers for probiotic bacteria was assessed and it was found that soybean sprouts were their best carrier (Świeca et al., 2017). In contrast, another study by the same author found that leguminous sprouts enriched in *Saccharomyces cerevisiae* var. *boulardii* are a new functional product, which is characterized by high health and nutritional properties (Świeca et al., 2019), hence the hypothesis about the combination of *Saccharomyces cerevisiae* var. *boulardii*, but with pseudocereal. This combination may change the composition and increase the nutraceutical effect of the raw material.

In connection with the above, the purpose of this work is to present the effect of modification of buckwheat sprouts *Fagopyrum esculentum* Moench by probiotic yeast *Saccharomyces cerevisiae* var. *boulardii* to change the composition of fatty acids that increase the health potential of this raw material.

MATERIAL AND METHODS

Buckwheat seeds were purchased from the PNOS SA in Ożarów Mazowiecki, Poland.

Preparation of control and probiotic-rich sprouts

Strain of *Saccharomyces cerevisiae* var. *boulardii* (confirmed microscopically by sequencing the region containing the 3 'end of 18S rDNA, ITS1, 5.8S rDNA, ITS2 and the 5' end of 26S rDNA and by biochemical tests) was maintained on YPG agar slants (BTL, Łódź, Poland) at 4°C. It was cultured on malt agar for 48 hours at 30°C. Colonies of yeast were collected in a sterile manner from Petri dishes. They were suspended in water and then used to inoculate the seeds. The standard curve that was established was then used to determine the number of yeast cells *Saccharomyces cerevisiae* var. *boulardii* in a suspension at a level of 1×10^7 ml^{-1} based on the OD value. For preparation of the inoculum, the cell concentration was estimated by optical density (OD) analysis

at 600 nm using a spectrophotometer Smart Spec Plus (Bio-Rad, USA).

Seeds were disinfected in 1% (v/v) sodium hypochlorite for 10 min, then drained and washed with distilled water until they reached a neutral pH. Then, they were soaked in *Saccharomyces cerevisiae* var. *boulardii* water suspension (1×10^7 CFU per 1 gram of seeds; S, soaked with probiotics) or distilled water (C, control). Buckwheat seeds were imbibed for 4 hours. The seeds (approximately 12.5 g per plate) were dark-germinated for three days in a growth chamber (SANYO MLR-350H) on Petri dishes (φ 125 mm) lined with absorbent paper (relative humidity 90%). Seedlings were sprayed daily with Milli-Q water. Sprouting was run at 30°C. After three days, sprouts were manually collected and rinsed with distilled water. Microbiological tests were carried out immediately.

The material (grains, control and probiotic-rich sprouts) was frozen and lyophilized in Labconco Freezone 1L Freeze Dry System for nutritional and prohealth analysis.

Microbiological quality

The following microbiological analyses were performed in accordance with European or Polish standards.

Total mesophilic bacteria. The total number of mesophilic bacteria was determined with the plate technique on nutrient agar, according to PN-EN ISO 4833-2:2013 (Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 2: Colony count at 30 degrees C by the surface plating technique.

Yeasts and molds. Yeast and molds were differentiated according to the morphology of colonies. Their numbers were determined using the plate technique on glucose, yeast extract and chloramphenicol agar according to PN-ISO 21527-1:2009 (Microbiology of food and anima..., n.d.).

Determination of crude fats

The fat content of lyophilisates: seeds, controls and modified sprouts was determined using the Avanti Soxtec System (Model 2055 Manual Extraction Unit; Foss Tecator, Höganäs, Sweden).

Fat extraction

The fat from the freeze-dried seeds, control and probiotic-rich sprouts was extracted using the procedure described by Folch et al. (1957).

Analysis of fatty acid composition

GC-FID determined the percentage fatty acid composition. Fatty acid methyl esters were obtained according to the AOCS Official Method Ce 2-66 (n.d.).

Chromatographic separation was performed using Hewlett-Packard 5890 II apparatus equipped with a Supelcowax 10 capillary column (30 m × 0.25 mm × 0.25 μm) operating at programmed temperature conditions: from 60°C, at 12°C/min to 200°C maintained for 25 min. The temperature of the injection chamber and the detector was 240°C. Fatty acids were identified by comparing retention times with standards (Supelco 37 Component FAME Mix), and the results were reported as weight percentages following integration and calculation using a ChemStation (Agilent Technologies). This analysis were carried out in triplicate.

Indexes of lipid quality

Based on the fatty acid composition data, the atherogenicity index (IA) and the thrombogenicity index (IT) were calculated. The following equations were applied:

$$IA = [C12{:}0 + 4 \times (C14{:}0) + C16{:}0] / (MUFA + PUFA) \quad (1)$$

$$IT = (C14{:}0 + C16{:}0 + C18{:}0) / [0.5 \times MUFA + 0.5\ (n\text{-}6) + 3 \times (n\text{-}3) + (n\text{-}3 / n\text{-}6)] \quad (2)$$

where PUFA stands for polyene fatty acids (in the IA equation it is the sum of *n*-6 and *n*-3 fatty acids) and MUFA stands for monoenoic fatty acids (Ulbricht and Southgate, 1991).

Other lipid indices expressing the ratio of polyene fatty acids to saturated fatty acids (PUFA / SFA), the sum of polyene and monoenoic fatty acids to saturated fatty acids (UFA / SFA), and the ratio of *n*-6 to *n*-3 (*n*-6 / *n*-3) were calculated (Sinkovič et al., 2020).

Statistical analysis

Statistical analysis of the data was performed using Statistica 10 (StatSoft, Tulsa, OK, USA). For statistical

analysis, one-way analysis of variance and intergroup differences was used by Tukey's HSD post-hoc test with a significance level of $P < 0.05$, $P < 0.01$ and $P < 0.001$. Significant differences were denoted with different superscript letters.

RESULTS AND DISCUSSION

Germination is a natural biological process that occurs in the seeds of pseudocereals such as *Fagopyrum esculentum* Moench, *Fagopyrum tataricum* (L.) Gaertn., *Amaranth caudatus* L. This process affects a number of changes in seeds, including fat degradation to free fatty acids (Guardianelli et al., 2019; Marton et al., 2010).

Microbiological analyses

The amount of TMB, yeast and mold in the controls and probiotic sprouts is shown in Table 1. It was proved that after 3 days of sprouting a single edible portion of sprouts enriched with probiotic yeast contained an amount classifying them as a probiotic product (6.5 log/100 g f.m.). Co-culture of *Saccharomyces cerevisiae* var. *boulardii* and buckwheat caused a slight increase in the total count of mesophilic bacteria. It may be suggested that probiotic yeast caused a loosening of seed structure, which promotes the growth of other microorganisms.

Table 1. Count of TMB, yeast and molds in the control and probiotic-rich sprouts

Buckwheat forms	Count of microorganisms log 10 CFU/g f.m.		
	total mesophilic bacteria	yeasts	molds
Control sprouts	$7.13^{a,***}$±0.02	$1.43^{a,***}$±0.15	$0^{a,***}$
Probiotic-rich sprouts	$7.99^{b,***}$ ±0.03	$4.53^{b,***}$±0.05	$0^{a,***}$

Mean values with different letters in the column are statistically significantly different (***, $P \leq 0.001$). "±" means standard deviation.

Total fat content

During the germination process, the total amount of fat increased in sprouts ($P < 0.001$). The fat content is shown in Figure 1. Compared to the dormant seeds the total fat content in the control sprouts and probiotic-rich sprouts was higher by c. 50%. There were no statistical differences between the control sprouts and those enriched with yeasts.

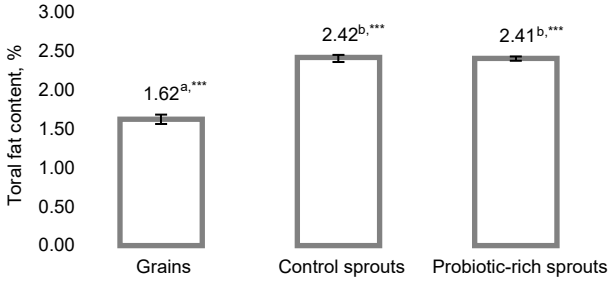

Fig. 1. Total fat content in different forms of buckwheat, %. Mean values with different letters above the bars are statistically significantly different (***, $P < 0.001$)

Fatty acid content and nutritional value of buckwheat seeds and sprouts

Table 2 presents the composition of fatty acids in extracts from lyophilized buckwheat seeds, as well as the control and probiotic-rich sprouts. Germination affected the change of profile and the percentage of individual fatty acids. In total, 13 fatty acids were identified in the seeds and 11 in the sprouts. Myristoleic and pentadecanoic acids were found in the seeds, but not in both types of sprouts. Saturated fats constitute 21.2% in seeds, whereas in sprouts they make up 16.84% (control) and 16.86% (modification), respectively, and unsaturated fats 78.48% ±0.01, respectively; 83.01% ±0.04 and 83.16% ±0.04 of the total fatty acid content.

The highest content of palmitic acid, oleic acid and linoleic acid was found in seeds and sprouts. These results in the case of seeds coincide with the data presented in other scientific studies (Gulpinar et al., 2012; Kim et al., 2004; Taira et al., 1986). It should be noted here that although both the sprouts and the seeds had the highest amount of the above acids, their quantity changed. The content of palmitic acid decreased significantly in control (up to 13.90%) and modified (up to 13.62%) sprouts. In contrast, the linoleic acid content increased both in the control (up to 40.19%) and in the modified form (up to 42.45%).

Table 2. The percentage composition and the ratio of fatty acids in different forms of buckwheat

Fatty acids	Buckwheat forms		
	grains	control sprouts	probiotic--rich sprouts
Saturated			
Myristic acid C14:0	$0.14^{a,**}$ ±0.01	$0.13^{a,**}$ ±0.01	$0.14^{a,**}$ ±0.01
Palmitic acid C16:0	$16.56^{c,*}$ ±0.03	$13.90^{b,*}$ ±0.01	$13.62^{a,*}$ ±0.06
Margaric acid C17:0	$0.31^{b,***}$ ±0.01	$0.07^{a,***}$ ±0.01	$0.08^{a,***}$ ±0.08
Stearic acid C18:0	$2.24^{b,***}$ ±0.02	$1.64^{a,***}$ ±0.02	$1.69^{a,***}$ ±0.03
Arachidic acid C20:0	$1.87^{c,**}$ ±0.01	$1.12^{a,**}$ ±0.01	$1.34^{b,**}$ ±0.02
Monoenoic			
Palmitoleic acid C16:1	$0.40^{a,*}$ ±0.02	$0.57^{b,*}$ ±0.03	$0.60^{b,*}$ ±0.01
Ginkgolic acid C17:1	$0.09^{b,**}$ ±0.01	$0.18^{c,**}$ ±0.01	$0^{a,**}$
Oleic acid C18:1	$39.95^{c,***}$ ±0.1	$36.18^{b,***}$ ±0.09	$33.55^{a,***}$ ±0.11
Eicosenoic acid C20:1	$4.18^{c,***}$ ±0.02	$3.10^{a,***}$ ±0.01	$3.59^{b,***}$ ±0.03
Polyenic			
Linoleic acid C18:2	$32.16^{a,***}$ ±0.11	$40.19^{b,***}$ ±0.15	$42.45^{c,***}$ ±0.08
α-linolenic acid C18:3	$1.64^{a,***}$ ±0.05	$2.84^{b,***}$ ±0.02	$2.93^{b,***}$ ±0.08
Saturated:monoenoic:polyenic			
	1:2.1:1.6	1:2.4:2.6	1:2.2:2.7

Mean values with different letters in the row are statistically significantly different, with significance indicated as follows: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. "±" means standard deviation.

In the literature, lipids of microbial origin are referred to as Single Cell Oil (SCO), and the microorganisms that produce them as oily. Among them we can distinguish bacteria, molds, microalgae and yeasts. Oleaginous microorganisms are defined as being capable of producing and accumulating more than 20% of dry cell substance. They accumulate lipids, which are deposited in so-called lipid droplets (LD). Yeast is the best producer of microbial fat. This is characterized by a fast growth rate and low nutritional requirements. On the other hand, the fatty acid composition can be modified by changing the culture conditions. Not all yeast species are able to synthesize more than 20% of fat in a dry cell substance (Klug and Daum, 2014; Kot et al., 2015; Patel et al., 2020).

Lipid synthesis can proceed in two ways: *ex novo* and *de novo*. *De novo* is based on obtaining fat from acetyl-CoA and malonyl-CoA molecules. On the other hand, *ex novo* from hydrophobic substrates is present in an environment containing phytochemicals, i.e. alkanes, free fatty acids. Subsequently, either directly or after prior hydrolysis, the cells can, for example, be incorporated into biotransformation processes (Kot et al., 2015).

Saccharomyces cerevisiae has not yet been identified in the literature as an oleaginous microorganism. This species is more often referred to as a non-oleaginous yeast. However, there are studies showing their effect on fatty acid production. The mechanisms that stand out here include *de novo* synthesis (Klug and Daum, 2014; Patel et al., 2020).

The fatty acids synthesized by oleaginous microorganisms are primarily myristic, palmitic, stearic, oleic, linoleic, linolenic and palmitoleic acids (Klug and Daum, 2014; Kot et al., 2015). When analyzing the results presented in this publication, it can be seen that there has been a change in some of the fatty acids mentioned. This mainly applies to linoleic and linolenic acids, which, when comparing the control with probiotic-rich sprouts, shows an increase in its amount. Changes in the proportion of individual fatty acids could result from the activity of yeast. This requires further research.

Table 3 shows nutritional information about lyophilisate buckwheat products. The ratio of *n*-6 / *n*-3 fatty acids was higher in seeds than in sprouts. This is related to the change in the fatty acid profile, where the statistically significant amount of saturated acids decreased, while the amount of unsaturated acids increased. Probiotic-rich sprouts contained the most polyene acids. This is associated primarily with an increase in linoleic

Table 3. Nutritional information of lyophilisated buckwheat *Fagopyrum esculentum* Moench, %

Buckwheat forms	Nutrition information							
	SFA	MUFA	PUFA	PUFA/SFA	UFA/SFA	*n*-6 / *n*-3 ratio	IA	IT
Grains	$21.2^{b,***}$ ±0.06	$44.68^{c,***}$ ±0.16	$33.8^{a,***}$ ±0.16	$1.59^{a,***}$ ±0	$3.7^{a,***}$ ±0	$19.68^{b,**}$ ±0.53	$0.22^{b,***}$ ±0	$0.44^{b,***}$ ±0
Control sprouts	$16.84^{a,***}$ ±0	$40.05^{b,***}$ ±0.03	$42.96^{b,***}$ ±0.08	$2.55^{b,***}$ ±0	$4.93^{b,***}$ ±0	$14.17^{a,**}$ ±0.05	$0.17^{a,***}$ ±0	$0.32^{a,***}$ ±0
Probiotic-rich sprouts	$16.86^{a,***}$ ±0.08	$37.8^{a,***}$ ±0.06	$45.38^{c,***}$ ±0	$2.69^{b,***}$ ±0	$4.93^{b,***}$ ±0.02	$14.49^{a,**}$ ±0,45	$0.17^{a,***}$ ±0	$0.32^{a,***}$ ±0

Mean values with different letters in the column are statistically significantly different, with significance indicated as follows: ***, $P < 0.001$; **, $P < 0.01$. "±" means standard deviation.

acid. The PUFA/SFA ratio in probiotic sprouts was higher than in the control sprouts and seeds. Table 2 also shows the ratio of saturated to monoenoic and polyenic acids.

The consumption of *n*-6 fatty acids may be associated with a reduced risk of cardiovascular disease. In the publication by Sacks et al. (2017), it was indicated that saturated fatty acids could be replaced in the diet by polyene acids to prevent cardiovascular disease. The publication by Kim et al. (2004) indicates that during the germination process, the content of polyene acids increases and the number of oleic acid decreases (Kim et al., 2004; Sacks et al., 2017; Wang, 2018).

From a nutritional point of view, lower IA and IT values are considered to be better and such values were observed in this study. It was found that the values of these indicators were lower in sprouts than in seeds.

In the study of Tartary buckwheat *Fagopyrum tataricum* (L.) Gaertn., it was shown that during the germination period the content of palmitic acid (14.6%, 15.8%), oleic acid (53.8%, 61.4%), stearic acid increased (2.6%, 6.7%), and the amount of linoleic acid (27.9%, 14.9%), eicosenoic acid (0.8%, 0.6%) decreased (Yiming et al., 2015). However, the study by Guardianelli et al. (2019) examined flour obtained from germinated seeds of *Amaranth caudatus* L. The fatty acid content was tested in amaranth flour: control, germinated for 18 hours (G18) and 24 hours (G24). Referring sample G24 to the control sample, it was noticed that the content of palmitic, linoleic, α-linolenic acid increased significantly and the content of stearic acid significantly decreased (Guardianelli et al., 2019). The above research indicated that the germination process changed the composition of fatty acids, which is similar to the results regarding common buckwheat, which is the subject of this publication.

CONCLUSIONS

During germination, the composition and profile of fatty acids changed. Statistically significant differences are observed between control and probiotic-rich sprouts (palmitic, arachidic, ginkgolic, oleic, eicosenoic, linoleic acid).

Modification with probiotic yeast *Saccharomyces cerevisiae* var. *boulardii* changed the ratio of fatty acids, and increasing the content of polyene acids, both linoleic and alpha-linolenic acid IA and IT, in buckwheat grains and sprouts was considered nutritionally optimal.

Summing up, it can be concluded that buckwheat sprouts modified with the addition of probiotic yeast may constitute a new functional product that can be used in the diet as an element of the prevention of cardiovascular diseases.

To the best of our knowledge, no report has been published so far on pseudocereal sprouts modified by the addition of a probiotic yeast. This study is therefore innovative.

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Starch and protein analysis in buckwheat (*Fagopyrum esculentum* Moench) sprouts enriched with probiotic yeast

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ABSTRACT

The study aimed to investigate the effect of modification of *Fagopyrum esculentum* Moench sprouts by probiotic yeast strain on their nutritional value related to protein and starch. For this purpose, an attempt was also made to 1) evaluate the digestibility of protein and starch and 2) examine anti-nutritional factors that may affect the digestibility of the indicated macronutrients. Probiotic-rich sprouts were characterized by the highest content of total protein, free amino acids and peptides, resistant starch, and total free sugars. In addition, the amino acid profile of buckwheat sprouts changed compared to seeds. Modified buckwheat sprouts were characterized by a higher content of sulfur amino acids compared to seeds and control sprouts. The highest methionine content was found in sprouts rich in probiotics, 4.65 ± 0.29 mg/g 16gN. The results show also that the modification reduced protein and starch digestibility by 7.4% and 4.2%, respectively, compared to the seed. The digestibility may have been influenced by anti-nutritional compounds contained in the raw material, i.e. condensed tannins, phenols and α-amylase inhibitor. The presented research gives a new direction for the use of the raw material of buckwheat sprouts.

1. Introduction

Common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* L. Gaertn.) are the two main cultivated species of buckwheat and are widespread in China, Russia, and some European countries. It belongs to the *Polygonaceae* family and is classified as a pseudocereal (Chen & Ruan, 2016; Zawadzka, Kobus-Cisowska, & Stachowiak, 2021; Zhu, 2016). Common buckwheat has been gaining more and more popularity in recent years due to its health benefits and the possibility of using it as a functional food. Buckwheat grain is characterized by a high protein content with a favorable amino acid composition, starch, and a low content of α-gliadin, which is essential for people who have celiac disease. The amino acid composition of buckwheat during maturation is relatively stable compared to changes in the amino acid composition of, for example, cereals. Buckwheat is characterized by a higher content of lysine (limiting amino acid) and a lower content of glutamic acid (amino acids of storage proteins) (Sytar, Chrastinová, et al., 2018; Zhou et al., 2018). In the case of pseudocereals, the nutritional value is mainly related to their proteins (Gorinstein et al., 2002).

Starch can make up more than 51%–70% of the dry matter. Therefore, the properties and structures of starch, such as nutritional, organoleptic, and textural properties, are critical to the quality of buckwheat food products (Perez-Rea & Antezana-Gomez, 2017; Zhu, 2016).

Digestibility is an important element in interpreting the quality of the nutrients that are digested, such as proteins. Therefore, the digestibility of the food should be improved (Joye, 2019). In one of the studies, the speed and degree of starch digestibility were determined under the influence of technological factors in buckwheat samples: cooked buckwheat, bread with flour or buckwheat seeds, and buckwheat pasta. In the samples of buckwheat, the content of resistant starch was low. The highest content was that of boiled buckwheat (6% based on total starch). The lowest content was found in bread with 70% buckwheat flour (0.9% of total starch-resistant starch) (Škrabanja & Kreft, 2016). Starch in buckwheat products may behave differently to that contained in other flour-based grain products. This may be due to the fact that buckwheat

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itself contains one or more ingredients that can inhibit the amylolytic degradation of starch, such as an amyloglucosidase inhibitor, condensed tannins. (Škrabanja & Kreft, 2016).

Sprouting can affect the digestibility of starch and protein. The digestibility may be increased as a result of this process. It has been found that the levels of anti-nutritional compounds present in cereals and pseudocereals are lowered, thereby increasing the levels of essential nutrients. This is a consequence of the activation of enzymes and their involvement in the synthesis of chemical compounds that improve nutritional quality (Brajdes & Vizireanu, 2012).

Probiotics have been incorporated into products such as dairy products and fermented vegetables. In recent years, new alternative matrices for probiotics such as chocolate have been developed (Possemiers, Marzorati, Verstraete, & Van de Wiele, 2010; Tripathi & Giri, 2014). The World Health Organization defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". In order to be labeled a probiotic, scientific evidence for the health benefits would have to be documented (*FAO Document Repository* -, n.d.). In a study by Swieca et al., 2019, legumes were used as carriers for probiotic yeasts. They were shown to contain large amounts of resistant starch that can be metabolized by gut microbiota and probiotics. It was found that this can positively affect the raw material itself (Swieca et al., 2019).

Products to which buckwheat has been added, as well as the raw material itself, are associated with health benefits (Giménez-Bastida & Zieliński, 2015). In the study by Swieca et al., 2019, legume sprouts enriched with *Saccharomyces cerevisiae* var. *boulardii* has been identified as a new functional food product (Swieca et al., 2019). In a study by Molska, Reguła, Rudzińska, & Świeca, 2020, the authors concluded that the modified buckwheat sprouts, by adding a probiotic yeast strain, can be considered a new functional product. A product that can be used as part of a diet to reduce the risk of cardiovascular diseases (Molska et al., 2020).

Hence, there are hypotheses that adding a probiotic strain may change the composition of the basic raw material. The study aimed to investigate the influence of buckwheat sprouts modification on their nutritional value related to protein and starch. For this purpose, the authors also made an attempt to 1) evaluate the digestibility of protein and starch, and 2) investigate anti-nutritional factors that may affect the digestibility of the indicated macronutrients.

2. Materials and methods

2.1. Buckwheat seeds

Fagopyrum esculentun Moench seeds were purchased from PNOS S.A. in Ożarów Mazowiecki. First, the seeds were disinfected with 1% (v/v) sodium hypochlorite (Sigma-Aldrich, USA) for 10 min. They were then filtered and washed with distilled water until they reached a neutral pH. They were then placed for 4 h in an aqueous suspension of *Saccharomyces cerevisiae* var. *boulardii* yeast cells at the level of 1×10^7 ml^{-1} on the basis of the OD value (probiotic-rich sprouts) or in distilled water (control sprouts). The germination process took place in the dark. It lasted for three days in a growth chamber (SANYO MLR-350H) in Petri dishes (φ 125 mm) lined with absorbent paper. The seedlings were sprayed with Milli-Q water daily. The sprouts were then harvested by hand and rinsed with distilled water. The material was frozen and freeze-dried (Molska et al., 2020).

2.2. Protein analysis

2.2.1. Protein fractionation and total protein content

Fagopyrum esculentum Moench buckwheat seeds and sprout lyophilisates were used for protein extraction with 0.1M borate buffer, pH 8.3, 1:30 (w/v) for 60 min at room temperature. After the process was completed, the protein fractions (albumin, globulin, prolamine, and glutelin) were separated based on the solubility criterion according to the method of Ribeiro, Teixeira, and Ferreira (2004) with slight modifications (Ribeiro et al., 2004).

Protein fractions were successively extracted from the freeze-dried buckwheat products and purified using appropriate extraction solutions. Albumin was extracted in water containing 10 mM $CaCl_2$ and 10 mM $MgCl_2$ (30 mg/g wet weight). Insoluble proteins were removed by centrifugation at 9000×*g* and 4 °C for 30 min. The resulting supernatant was the albumin fraction. The pellet was then resuspended in 100 mM Tris-HCl, pH 7.5 buffer containing 10% (w/v) NaCl, 10 mM ethylenediaminetetraacetic acid (EDTA), (30 ml/g dry solids). Dissolved globulins were obtained by centrifugation at 9000×*g* and 4 °C for 30min. The obtained supernatant was the globulin fraction. The resulting precipitate was suspended in methanol, then shaken for 1 h. The prolamine fraction was obtained by centrifugation at 9000×*g* and 4 °C for 30 min. The procedure was repeated sequentially. This was done in order to obtain a glutelin fraction from a pellet containing insoluble material. The pellet was then re-suspended in 50 mM Tris-HCl buffer, pH 10, containing (v/v) β -mercaptoethanol and 1% (w/v) SDS (5 ml/g dry weight of pellet). The resulting suspension was stirred at room temperature for 30 min and centrifuged at 9000×*g* for 30 min.

According to the procedure, the total protein content (TP) was calculated as the sum of the protein fractions (Bradford, 1976).

2.3. Free amino acids and peptides

Using the methodology described by Periago, Ros, Martínez, and Rincón (1996), soluble protein and non-protein nitrogen (peptides and a fraction rich in free amino acids) were measured. A triple extraction of 25 ml of 0.02mol dm^{-3} NaOH was performed for 60 min, and three extracts were collected. First, the insoluble material was removed by centrifugation at 8700 RCF for 20 min, then the soluble protein was measured according to the procedure described by Lowry (1951), with bovine albumin as the standard. The obtained supernatant was mixed with 20 ml of 30% TCA, and the mixture was stirred for 15 min at 4 °C. Then, the protein was removed by centrifugation at 8700 RCF for 15 min. Finally, non-protein nitrogen was determined using the ninhydrin method (Sun et al., 2006) and expressed as the leucine equivalent in mg g^{-1} d.m (Lowry, Rosebrough, Farr, & Randall, 1951; Periago et al., 1996; S.-W. Sun, Lin, Weng, & Chen, 2006).

2.4. Amino acid profile of proteins

After acid hydrolysis (110 °C, 23h), the amino acid composition was determined ("AOAC SMPR 2014.013," 2015). Amino acid dilution and derivatization were performed (with AccQ•Tag reagents, No. 186003836, Waters) according to the manufacturers' protocol after sample evaporation. The samples were analyzed by ultra-efficient liquid chromatography (UPLC, Shimadzu Nexera 2.0, Kyoto, Japan) equipped with a binary solvent manager, autosampler, column heater, and PDA detector (Kyoto, Japan). An AccQ-Tag Ultra C18 1.7 μm (2.1 mm i.d. x 100 mm, 1.7 μm particles, Waters) was used as the separating column. The mobile phase flow rate was kept at 0.6 ml/min, and the column temperature was set to 55 °C. A non-linear separation gradient was created by mixing 5% and 100% AccQ • Tag Ultra (Waters) solvent. One microliter of the sample was injected for analysis. The PDA detector was set at 260 nm, with a sampling rate of 20 points/sec. Amino acid quantification was performed with amino acid standards which contained 2.5 μmol/ml for each amino acid in 0.1 mol/L HCl (088122, Waters). Standards were diluted 25 times with ultrapure water. The diluted standard (10, 20 or 60 μl) was mixed with 70 μl of borate buffer and 20 μl of AccQ•Tag reagents, performing standard amino acid derivatization. One microliter of sample obtained was used for UPLC analysis, and was injected 5 times to prepare the calibration curve with the LabSolution program (Shimadzu Corp., Kyoto, Japan). The amino acid content is expressed in g/16 g N (corresponding to g/100g protein).

Tryptophan was determined spectrophotometrically (*AOAC 988.15-1988, Tryptophan in Foods and Food and Feed Ingredie - $14.30: AOAC Official Method*, n.d.), with a HALO DB-20 UV–vis Double beam Spectrophotometer, Dynamica.

3. Protein digestibility

3.1. In vitro digestion

In vitro digestion was performed as described by Minekus et al. with some modifications by Sęczyk et al. (Minekus et al., 2014; Sęczyk, Świeca, & Gawlik-Dziki, 2015).

Simulated gastrointestinal (IE) supernatants were stored in the dark at −20 °C until being analyzed.

Blanks were prepared under the same conditions (not using *Fagopyrum esculentum* lyophilisates). Pellets were frozen, lyophilized, and stored in the dark at −20 °C until analysis.

3.2. Protein digestibility

Based on the protein content before (TP; sum of protein fractions determined according to the procedure described in 2.2.1.) and after *in vitro* digestion, the protein digestibility (PD) was assessed. In order to determine the protein content after the digestion procedure, the pellets were fractionated according to the procedure described by Chang at al. 2014 (Chang, Ismail, Yanagita, Esa, & Baharuldin, 2014).

The content of albumin and globulin was determined as the proteins present in the supernatants obtained after *in vitro* digestion (the digestive system components were corrected). Proteins after *in vitro* digestion were determined as the sum of all fractions.

Protein digestibility was calculated according to the following formula:

$$PD[\%] = 100\% - ((Pa/Pb) \times 100\%);$$

where PD is the *in vitro* digestibility of proteins, Pb is the protein content before *in vitro* digestion, and Pa is the protein content after *in vitro* digestion.

4. Analysis of free sugars

Free sugars were analyzed using a YMC-Pack Polyamine II 5 μm (250 4.6 mm) column from YMC Co., Ltd. (Kyotyo, Japan). Twenty milliliters of a 20% ethanol solution were added to 1 g of freeze-dried seeds and buckwheat sprouts and shaken for 60 min in a 35 °C water bath. The samples were centrifuged at 5000g for 5 min. The supernatant was filtered using a Sep-Pak NH2 solid phase extraction cartridge (Waters, USA). Secondly, 1.0 ml of the filtrate was evaporated to dryness in a 50 °C dry bath by blowing with N_2 gas. What was left was dissolved in 0.3 ml of water. 20 μl was injected into an HPLC equipped with a Waters 510 pump, Waters 410 RI detector, and Waters 746 Integrator. The operating conditions were as follows: column temperature 35 °C; flow rate 0.7 ml min 1; mobile phase acetonitrile: water (65:35, v/v); detector temperature 39 °C. The simple sugars and oligosaccharides were obtained from Sigma (St. Louis, USA) (Kim, Kim, & Park, 2004).

5. Starch

5.1. Total starch (TS)

Total starch content in the flour was determined after dispersion of the starch granules in 2M KOH according to Goñi, García-Alonso, & Saura-calixto, 1997 (Goñi et al., 1997). Glucose content was determined using the standard dinitrosalicylic acid (DNSA) method (Miller, 1959). Total starch was calculated as glucose × 0.9.

5.2. Resistant starch and potentially available starch

The resistant (RS) and potentially bioavailable (AS) starch content was analyzed on the basis of results obtained after simulated gastrointestinal digestion. Resistant starch (RS) was calculated as glucose x 0.9. The potentially bioavailable starch (AS) content was calculated as the differences between TS and RS.

5.3. Total free sugars content

The sugar profile was determined using the enzymatic-spectrophotometric method. First, the samples were dissolved in warm water. Next, fat and precipitated proteins were separated by filtration. The filtrate was treated with enzymes and biochemicals added simultaneously but acting in a specific sequence. The amount of NADPH formed was measured spectrophotometrically at a wavelength of 340 nm and converted to the content of individual sugars. The procedure was performed in accordance with the Megazyme assay protocol (Sucrose/D-Fructose/D-Glucose Assay Kit) and a publication by Steegmans et all. 2004 (Steegmans, Iliaens, & Hoebregs, 2004).

5.4. In vitro starch digestion rate and expected glycemic index

The digestive kinetics and the expected glycemic index (eGI) of buckwheat were calculated according to a procedure established by Goñi et al., 1997 (Goñi et al., 1997). A non-linear model was applied according to the equation [$C = C_\infty/(1\text{-}e^{-kt})$], where C, C_∞, and k were each time the degree of hydrolysis, the maximum degree of hydrolysis, and the corresponding kinetic constant. This was used to describe the kinetics of starch hydrolysis. On the other hand, the expected glycemic index (eGI) was calculated using an equation proposed by Granfeldt, Björck, Drews, & Tovar, 1992 (Granfeldt et al., 1992). The glycemic load (GL) is calculated indirectly as the glycemic index (eGI) times the weight of available carbohydrates. The GL for 100g of buckwheat lyophilisate was calculated from the equation (Venn et al., 2006).

$$GL = (P \times GI)100$$

where:

GL - glycemic load

P - proportion of available carbohydrates in the portion of food (g).

6. Starch digestibility

In vitro digestion was performed as described by Minekus et al. with some modifications (Minekus et al., 2014; Sęczyk et al., 2015), which were described in point 3.1.

The i*n vitro* digestibility of starch was evaluated on the basis of total starch content (TS) and resistant starch (RS) determined after digestion *in vitro* (Świeca, Baraniak, & Gawlik-Dziki, 2013).

$$SD[\%] = 100\% - ((RS/TS) \times 100\%)$$

Where: SD is the *in vitro* digestibility of starch, TS the total starch content, and RS is the resistant starch content.

7. Factors affecting nutrient digestibility

7.1. Activity of hydrolases inhibitors

7.1.1. Extraction of hydrolases inhibitors

For extraction, 100 mg of buckwheat sprouts were suspended in 4 mL of 50 mM phosphate buffer, pH 7.6, then stirred for 2 h at 4 °C and centrifuged at 9000×*g* for 20 min. The supernatant was used for further analysis.

7.1.2. Trypsin inhibitor activity

The trypsin inhibitor activity (TIA) was determined using α-N-benzoyl-DL-arginine-p-nitroanilidehydrochloride (BAPNA) as the substrate (Kakade, Rackis, McGhee, & Puski, 1974). Pre-incubation was in a solution containing 0.25 ml of inhibitor solution, 0.25 ml of porcine pancreatic trypsin at 37 °C for 15 min. Then 0.5 ml of 0.5 mM BAPNA was added and immediately vortexed to start the reaction. After 10 min of incubation, 0.5 ml of 30% acetic acid (v/v) was added to terminate the reaction. The reaction mixture was centrifuged at 8000×*g* for 5 min. The residual trypisin activity was then measured by absorbance at 410 nm due to p-nitroaniline. TIA is expressed as trypsin inhibitor units per milligram of dry sample (d.m.). An increase of 0.001 absorbance units at 410 nm of the reaction mixture was defined as one trypsin unit.

7.1.3. Activity of α -amylase inhibitor

Alpha-amylase inhibitor activity was measured according to the modified method of Deshpande et al., 1984 (Deshpande & Cheryan, 1984). Porcine pancreatic α-amylase (220 U/ml) was dissolved in 100 mM phosphate buffer (containing 20 mM $CaCl_2$, pH 5.6). The alpha-amylase inhibitory activity was then measured. For this purpose, a mixture of 0.25 ml of alpha-amylase solution and 0.25 ml of extracted **α**-amylase inhibitor was first incubated in a water bath at 40 °C for 15 min. Secondly, 0.5 ml of 1% (w/v) soluble starch (dissolved in 20 mM sodium phosphate buffer containing 6 mM NaCl, pH 7) was added. After 10 min, the reaction was stopped by adding 1 ml of 3,5-dinitrosalicylic acid and the mixture was heated in a boiling water bath for 10 min. The mixture was then made up to 12 ml with doubly distilled water. The obtained results were compared with the activity of the same amount of enzyme without the inhibitor. The amount of inhibitor that reduces the activity of the enzyme by one unit was defined as one inhibitory unit. In contrast, one unit of α-amylase activity was defined as an increase in absorbance of 0.001 under test conditions.

7.1.4. Amyloglucosidase inhibitor activity

The amyloglucosidase inhibitor activity was determined using an α-Glucosidase Activity Assay Kit (MAK123-1 KT).

7.1.5. Pepsin inhibitor activity

Pepsin inhibitor activity was measured according to the modified method of Anson (1938). One unit of Pepsin produced a ΔA_{280} of 0.001 per minute at pH 2.0 at 37 °C measured as trichloroacetic acid (TCA)--soluble products using hemoglobin as the substrate (Anson, 1938).

7.2. Total phenolics content

Using a 3-step extraction method, 0.5 g were extracted. In the first stage of the test, 5 ml of 50% methanol was extracted for 30 min at room temperature using a multi-rotator (RS-60, Biosan) (300 rpm). Then, the samples were centrifuged (6000g, 15min). The resulting precipitates were re-extracted with 5 ml of 1% HCl in 50% methanol, and then finally with 5 ml of 80% acetone. The collected supernatants from all analysis steps were pooled and stored for use in further analysis steps. Phenolics were also determined before and after *in vitro* digestion (Bochnak-Niedźwiecka, Szymanowska, & Świeca, 2020).

Using the Folin-Ciocalteau reagent, the amount of total phenolic compounds was determined (Singleton, Orthofer, & Lamuela-Raventós, 1999). 0.5 ml of H_2O and 2 ml of Folin-Ciocalteau reagent (1:5 H_2O) were added to 0.5 ml of extract obtained after *in vitro* digestion. After 3 min, 10 ml of 10% Na_2CO_3 was added, and the contents were mixed and allowed to stand for 30 min. The absorbance at 725 nm was measured. The total amount of phenols was expressed as the gallic acid equivalent (GAE) in mg per g dry mass of sprouts (d.m.).

7.3. Condensed tannins content

According to the method described by Sun, Ricardo-da-Silva, & Spranger, 1998, the content of condensed tannins in the raw material was determined (B. Sun et al., 1998). 1 ml of a freshly prepared vanillin reagent (1% (w/v) vanillin in ice-cold acetic acid: HCl (92:8) solution) was added to the obtained extract (0.2 ml) after *in vitro* digestion. This was followed by incubation, after which the absorbance at 510 nm was measured for 20 min at 30 °C. The content of condensed tannin was expressed as the equivalent (+) catechin (CE) in mg per g dry mass of sprouts (DM.).

8. Statistical analysis

All experimental results were mean ± S.D. of three independent experiments (n = 9). One-way analysis of variance (ANOVA) and Tukey's post-hoc test were used to compare groups (seeds, as well as control and elicited sprouts) (STATISTICA 10, StatSoft, Inc., Tulsa, USA). Differences were considered significant at $p < 0.05$. The correlation between the tested parameters was determined using principal component analysis (PCA).

9. Results and discussion

9.1. PCA analysis

PCA was applied to observe possible clusters in the pâté samples under analysis. The analysis was performed for the following parameters: Trypsin inhibitor; Free amino acids and peptides; α-Amylase inhibitors; Pepsin inhibitor; Amyloglucosidase inhibitor; Condensed tannins; Available starch; Starch digestibility; Total starch; Protein digestibility; Total phenolic compounds; Resistant starch. The first two principal factors accounted for 82.86% (PF1 = 66.13% and PF2 = 16.73%) of the total variation. The plot of variables (Fig. 1a) shows that negatively correlated variables point to opposite sides of the plot, and positively correlated variables point to the same side of the plot. Negatively correlated variables are placed on opposite sides of the plot origin (opposite quadrants), while positively correlated variables are grouped together. The distance between the origin and the variables measures the quality of the variables on the factor map. Consequently, variables that are far from the origin are well represented on the factor map.

Positively correlated variables are, e.g., protein digestibility and trypsin inhibitor, protein inhibitors; starch digestibility and resistant starch. Negatively correlated variables are e.g. protein digestibility and condensed tannins (CTC), total phenolic compounds (TPC).

The graph of the scattering of objects in the space defined by the first two main components (Fig. 1b) also provides interesting information. There are three quite compact clusters of points depicting individual groups, among which the probiotic rich group forms the strictest cluster.

9.2. Protein, amino acid profile, and free amino acids and peptides

The Tables 1 and 2 show the protein, amino acid profile, and free amino acids and peptides. The highest total protein content was found in the probiotic rich sprouts group. An increase of approx. 22% was observed in relation to the seeds. This increase is mainly due to the higher level of glutelins which, together with globulins, constitutes the dominant fraction of buckwheat proteins. In the seeds and control sprouts, the protein level was comparable. The highest protein digestibility was found for albumin and globulin (86%–90% respectively in the control and probiotic-rich sprouts). The lowest digestibility was found for the prolamine fraction. It was noted that the digestibility of total protein in the probiotic-rich sprouts was 6% and 4% lower than the seeds and control sprouts respectively, though the differences are negligible. Despite the lower digestibility, the amounts of potentially bioavailable proteins are highest in the probiotic-rich sprouts (the lower availability is compensated for by the higher content).

In our study, the total amount of protein is comparable to that found

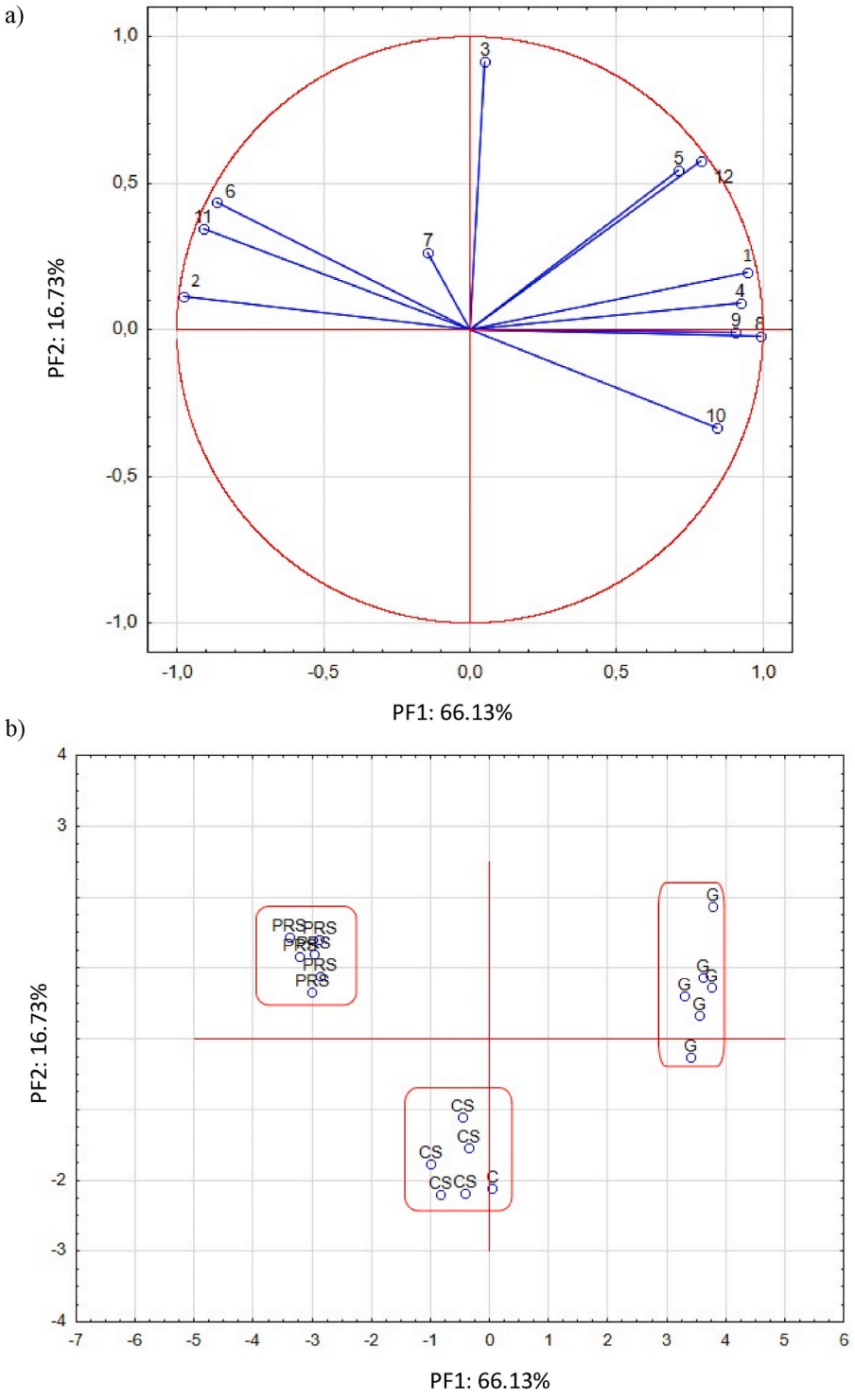

Fig. 1. Principal components analysis (PCA) for tested parameters;
1-Trypsin inhibitor (TI) [kIU/100 mg dw]; 2- Free amino acids and peptides [mg/g dw]; 3- α -amylase inhibitor [kIU/100 mg dw]; 4- Pepsin inhibitor (PI) [kIU/100 mg dw]; 5- Amyloglucosidase inhibitor (AMG) [kIU/100 mg dw]; 6- Condensed tannins (CTC) [mg/g dw]; 7- Available starch (AS) [mg/g f. m.]; 8- Starch digestibility [%]; 9- Total starch (TS) [mg/g f.m.]; 10- Protein digestibility [%]; 11- Total phenolic compounds (TPC) [[mg GAE/g dw]; 12 - Resistant starch (RS) [mg/g f.m.].

by other researchers. A protein concentration of 12–19% is generally observed in buckwheat grains. It is higher than that determined by Schoenlechner, 2016 (Schoenlechner, 2016). The protein content is highly dependent on the species, various environmental factors, and crop management practices. This pseudocereal contains a small amount of protein compared to the seeds of legumes, e.g., by 23.6% in beans (*Phaseolus vulgaris*) and by 36.1% in soybeans (*Glycine max*) (Schoenlechner, 2016; Zhu, 2016).

Buckwheat is considered a highly nutritious pseudocereal due to its protein profile. The amount of free amino acids and peptides increased in the control sprouts and probiotic-rich sprouts by 33.1% and 64.6%, respectively. Statistically significant differences were noted in the amount of amino acids, i.e. histidine, glutaminc acid, isoleucine, tyrosine, and sulfur amino acids (methionine + cysteine) (comparison of seeds to sprouts). On the other hand, a statistically significant increase in the content of aspartic acid, glutamic acid, tyrosine and sulfur amino acids (methionine + cysteine) was observed in probiotic rich sprouts compared to control sprouts. The highest amount of methionine was found in sprouts rich in probiotics 4.65 ± 0.29 mg/g 16gN. In previous publications, it was noted that methionine accumulates more intensively

Table 1
Protein fractions and free amino acids content in seeds and sprouts of buckwheat.

		Grains	Control sprouts	Probiotic-rich sprouts
Proteins [mg/g d.w.]	Albumins	19.2 ± 1.40^{b}	12.7 ± 0.70^{a}	12.5 ± 0.69^{a}
	Globulins	40.3 ± 1.90^{bc}	32.9 ± 2.90^{a}	38.0 ± 3.20^{ab}
	Prolamins	24.8 ± 2.00^{a}	24.05 ± 2.80^{a}	32.5 ± 2.60^{b}
	Glutelins	34.5 ± 2.40^{a}	46.4 ± 2.30^{b}	61.3 ± 2.20^{c}
	Total	119 ± 9.30^{a}	116 ± 7.80^{a}	144 ± 9.20^{b}
Free amino acids and peptides [mg/g dw]		5.28 ± 0.14^{a}	7.03 ± 0.07^{b}	8.69 ± 0.27^{c}

Mean values with different letters in the row are statistically significantly different, with significance indicated as $p < 0.05$. "±" means standard deviation.

Table 2
Amino acids content in seeds and sprouts of buckwheat.

		Grains (G)	Control sprouts (CS)	Probiotic-rich sprouts (PRS)
Amino acids [g/16gN]	His	4.26 ± 0.25^{b}	4.21 ± 0.02^{b}	3.13 ± 0.02^{a}
	Ser	6.54 ± 0.72^{a}	6.00 ± 0.65^{a}	6.82 ± 0.3^{a}
	Arg	11.65 ± 0.58^{a}	10.22 ± 0.18^{a}	10.41 ± 0.38^{a}
	Gly	7.72 ± 0.56^{a}	6.96 ± 0.44^{a}	7.62 ± 0.49^{a}
	Asp	14.18 ± 0.41^{b}	11.41 ± 0.12^{a}	13.65 ± 0.67^{b}
	Glu	26.76 ± 0.41^{c}	20.53 ± 0.75^{a}	23.98 ± 0.4^{b}
	Thr	5.42 ± 0.28^{a}	4.61 ± 0.71^{a}	5.13 ± 0.65^{a}
	Ala	6.14 ± 0.12^{a}	5.39 ± 0.54^{a}	6.49 ± 0.51^{a}
	Pro	5.04 ± 0.23^{a}	4.70 ± 0.43^{a}	5.60 ± 0.47^{a}
	Lys	6.14 ± 0.18^{a}	5.27 ± 0.76^{a}	5.80 ± 0.21^{a}
	Val	5.53 ± 0.16^{a}	4.96 ± 0.75^{a}	4.02 ± 0.28^{a}
	Ile	4.83 ± 0.14^{b}	3.91 ± 0.42^{ab}	3.36 ± 0.12^{a}
	Leu	8.55 ± 0.46^{a}	7.76 ± 0.45^{a}	8.56 ± 0.3^{a}
	Phe	5.22 ± 0.14^{a}	5.25 ± 0.55^{a}	5.63 ± 0.3^{a}
	Tyr	0.37 ± 0.04^{a}	0.85 ± 0.02^{b}	1.71 ± 0.14^{c}
	Met	1.04 ± 0.04^{a}	3.39 ± 0.14^{b}	4.65 ± 0.29^{c}
	Cys	1.51 ± 0.05^{b}	0.34 ± 0.06^{a}	0.48 ± 0.06^{a}
	Trp	1.63 ± 0.06^{a}	1.72 ± 0.07^{a}	1.84 ± 0.01^{a}
	Endogenic	68.25 ± 2.48^{b}	56.18 ± 2.61^{a}	66.34 ± 2.92^{ab}
	Exogenous	54.28 ± 2.18^{a}	51.30 ± 2.47^{a}	52.53 ± 2.56^{a}
	Total	122.53 ± 4.65^{a}	107.49 ± 5.08^{a}	118.87 ± 5.48^{a}

His- Histidine; Ser-Serine; Arg-Arginine; Gly-Glycine; Asp-Aspartic acid; Glu-Glutamic acid; Thr-Threonine; Ala-Alanine; Pro-Proline; Lys-Lysine; Val-Valine; Ile-Isoleucine; Leu-Leucine; Phe-Phenylalanine; Tyr-Tyrosine; Met-Methionine; Cys-Cysteine; Trp-Tryptophan.
Mean values with different letters in the row are statistically significantly different, with significance indicated as $p < 0.05$. "±" means standard deviation.

in the early growth phase than cysteine. It has been pointed out that the metabolism of methionine is subject to strict regulatory control and may be regulated differently in different buckwheat species (Hesse, Kreft, Maimann, Zeh, & Hoefgen, 2004; Sytar, Biel, Smetanska, & Brestic, 2018). The statistically significant increase in methionine in the probiotic-rich sprouts could have been caused by yeast present in the germination process. Yeast is capable of producing this amino acid. In a publication by Hesse et al., 2004, the first step in the synthesis of Met *de novo* in plants is the formation of cysteine and O-phosphohomoserine as a cystathionine thioether from the substances. The reaction is catalyzed by cystathionine synthase and involves a transsulfuration process via a γ-replacement reaction. At this stage, the Met synthase is separated from other amino acids belonging to the aspartate family. This is because Met is linked to the sulfur assimilation pathway. It is worth mentioning that the carbon precursor of Met synthesis differs from that of bacteria and yeasts. In yeast, Met is synthesized by direct sulfuration of O-acetylhomoserine (Hesse et al., 2004).

Bonafaccia, Marocchini, & Kreft, 2003 showed that buckwheat is characterized by a balanced amino acid composition, with a high proportion of exogenous amino acids such as leucine and lysine (6.92, 5.84 and 7.11, respectively, 6.18g/100g of protein in common buckwheat and Tartary) (Bonafaccia et al., 2003).

Data on the composition and content of amino acids in the buckwheat grain *Fagopyrum esculentum*, depending on their origin, show varietal differences in the content of amino acids (Sytar, Biel, et al., 2018). Fourteen amino acids have been identified in the sprouts: among others, lysine, aspartic acid, and glutamic acid (whose content increased significantly) (Sytar, Brestic, Zivcak, & Tran, 2016).

9.3. Protein and starch digestibility

The digestibility of buckwheat protein is low due to anti-nutritional factors, including tannins and protease inhibitors. On the other hand, the lack of gluten in this pseudocereal makes it possible to use it in gluten-free diets (Sytar et al., 2016).

Table 4 shows the protein starch digestibility and factors influencing it. In the presented study, protein digestibility decreased in the germination process. It decreased in the control and probiotic-rich sprouts by 2.5% and 7.4%, respectively. The situation is similar with the trypsin and pepsin inhibitor. The trypsin inhibitor and pepsin values decreased by 41.6% and 45.6%, respectively, in the probiotic-rich sprouts compared to the seeds.

There was an increase in total phenols during the sprouting process. A statistically significant difference can be noticed when comparing the control sprouts with the probiotic-rich sprouts. The total phenols content (TPC) in the probiotic-rich sprouts increased by 130% compared to the seeds. The statistically high content of condensed tannins and phenols compared to the seeds could have reduced the protein digestibility. Despite the high content of trypsin inhibitor and proteases from among the tested samples, the seeds showed the highest protein digestibility.

In buckwheat, protein digestibility is similar to millet, where protein digestibility is lower than other cereals. This is potentially due to the binding of polyphenols to proteins; interactions between proteins and phenolic compounds slow down the digestion of proteins in the small and large intestine. (Luthar, Zhou, Golob, & Germ, 2020). Qian, Rayas-Duarte, and Grant (1998) reported that compared to maize and wheat, buckwheat starch has a higher degree of hydrolysis, both in hydrochloric acid solution and as a result of enzymatic digestion using porcine pancreatic α-amylase. However, a publication by Kreft et al. (2002) described a slow rate of glucose release from buckwheat and a relatively high share of resistant starch. Consequently, this makes it a suitable component of diabetic diets (Kreft & Skrabanja, 2002; Qian et al., 1998).

Fig. 2 shows the kinetics of starch hydrolysis. The digestion rate of starch was expressed as a percentage of total starch hydrolyzed at different times. It is noted that during the first 60 min a large amount of

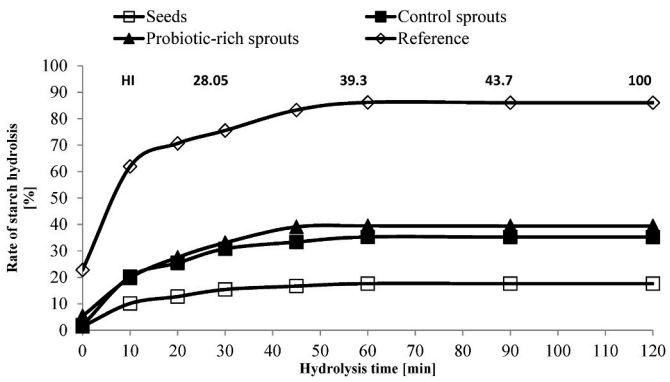

Fig. 2. Kinetics of starch hydrolysis.

glucose is released, while from 50 to 60 min a gradual decrease in rate is shown.

The calculated hydrolysis index (HI) and the predicted glycemic index (eGI) of the sprouts were significantly higher than those determined for the lyophilisate of dry seeds. However, when comparing the obtained results to the glycemic index tables, the obtained values belong to the low glycemic index. According to The University of Sydney, the seed glycemic index (eGI) is in the range of 49–63 and the glycemic load of 15–19 (*GI Search – Glycemic Index*, n.d.).

Significant differences in eGI and glycemic load (GL) were observed between the control sprouts and the probiotic-rich sprouts. Table 3 presents information on the carbohydrate content in buckwheat. There were statistically significant differences between seeds and sprouts in the amount of free sugars. Also, statistically significant changes were observed in the case of glucose and fructose. This is related to the germination process, which changed the content of induced compounds.

The increase in the free sugar content in the probiotic-rich sprouts compared to the seeds was 260.1%. There are differences between the content of glucose, fructose and sucrose between control sprouts and modified sprouts. Their higher values were tested in probiotic-rich sprouts. The glucose content increased by 310% in the control sprouts compared to the seeds and by 630% in the probiotic-rich sprouts. In the case of fructose, the increase in its content in control sprouts and sprouts rich in probiotics was 57.9% and 221.1%, respectively, compared to seeds.

In the control sprouts and probiotic-rich sprouts, the digestibility of starch decreased during the sprouting process by 2.52% and 4.24%, respectively. The activity of α-amylase and amyloglucosidase decreased statistically significantly in the control sprouts. The probiotic-rich sprouts showed no statistically significant differences from the seeds.

Since the digestibility of starch is often related to the activity of the α-amylase inhibitor, these two parameters are presented together to indicate some possible dependencies (Świeca et al., 2013). The seeds showed the highest digestibility of starch and the lowest ones showed probiotic-rich sprouts (Table 4). The α-amylase inhibitor activity in the control sprouts was lower than in the seeds and probiotic-rich sprouts (respectively lower value by 20.83%, 22.45%). The seeds showed the highest activity of an amyloglucosidase inhibitor by 100%, with 60.7% in the probiotic-rich sprouts.

In addition to the aforementioned amyloglucosidase and α-amylase, it is noted that the presence of tannins, phytic acid, and protease inhibitors reduces the susceptibility of enzymes and the digestibility of starch (Zhu, 2016).

The changes in nutrients (e.g. starch) during germination depend on the process conditions, such as time and temperature (Świeca et al., 2013; Zhang et.al., 2015). Additionally, the growth conditions during sprouting may have a significant impact on the composition of secondary metabolites with potential health-promoting properties. For example, there is a statistically significant decrease in the amount of total resistant starch during the sprouting process, which may be related to the use of starch as an energy source. Scientific research has shown that the activity of α -amylase inhibitors plays a key role in the digestibility of starch. On the other hand, researchers have shown an increase in the activity of these inhibitors in response to abiotic stresses such as drought or salinity. It is worth mentioning that the factors used to modify the growth of sprouts cause osmotic stress, which in turn causes the starch in the samples to accumulate in granules, which are weakly affected by hydrolytic enzymes. As a consequence, this causes indigestion or restriction of starch digestion (Hoover & Zhou, 2003; Świeca et al., 2013).

10. Conclusion

Compared to the control sprouts, the sprouts rich in probiotics were characterized by the highest content of e.g. total protein, free amino acids and peptides, and resistant starch. The probiotic-rich sprouts were characterized by the highest content of endogenous amino acids and methionine (an essential amino acid).

Compared to the seeds, it is noted that the digestibility of the protein and starch in the sprouts has changed. A number of factors and compounds contained in the raw buckwheat affect the digestibility of these ingredients. It is worth noting that despite the lower digestibility, the amounts of potentially bioavailable proteins are highest in probiotic-

Table 3
Carbohydrate in seeds and sprouts of buckwheat.

	Grains	Control sprouts	Probiotic-rich sprouts
Total starch (TS) [mg/g f. m.]	572.09 ± 20.13^{b}	543.32 ± 24.6^{a}	525.11 ± 12.24^{a}
Resistant starch (RS) [mg/g f.m.]	109.20 ± 0.01^{c}	87.83 ± 0.01^{a}	93.69 ± 0.01^{b}
Available starch (AS) [mg/g f.m.]	450.75 ± 0.01^{c}	445.73 ± 0.01^{b}	421.02 ± 0.01^{a}
Potentially available sugars [mg/g]	515.53 ± 32.15^{a}	514.55 ± 13.01^{a}	521.1 ± 30.58^{a}
Expected glycemic index (eGI)	28.2 ± 1.02^{a}	42.10 ± 1.28^{b}	45.87 ± 1.46^{c}
Glycemic load (GL)	15.9 ± 0.01^{a}	22.29 ± 0.01^{b}	23.35 ± 0.06^{c}
Glucose [mg/g]	0.20 ± 0.01^{a}	0.82 ± 0.02^{b}	1.46 ± 0.14^{c}
Fructose [mg/g]	0.19 ± 0.03^{a}	0.30 ± 0.01^{b}	0.61 ± 0.02^{c}
Saccharose [mg/g]	1.09 ± 0.08^{a}	0.81 ± 0.03^{a}	3.26 ± 0.24^{b}
Total free sugars [mg/g]	1.48 ± 0.12^{a}	1.93 ± 0.06^{b}	5.33 ± 0.39^{c}

Mean values with different letters in the row are statistically significantly different, with significance indicated as $p < 0.05$. "±" means standard deviation.

Table 4
Effect of anti-nutritional factors on starch and protein digestibility.

Parameters	Grains	Control sprouts	Probiotic-rich sprouts
Protein digestibility [%]	75.59 ± 3.02^{c}	73.67 ± 4.50^{b}	70.00 ± 2.83^{a}
Trypsin inhibitor (TI) [kIU/100 mg dw]	83.16 ± 5.23^{c}	56.05 ± 3.50^{b}	48.57 ± 3.03^{a}
Pepsin inhibitor (PI) [kIU/100 mg dw]	6.60 ± 0.63^{c}	4.5 ± 0.80^{b}	3.59 ± 0.46^{a}
Condensed tannins (CTC) [mg/g dw]	2.44 ± 0.29^{a}	2.93 ± 0.24^{b}	5.52 ± 0.29^{c}
Total phenolic compounds (TPC) [mg GAE/g dw]	10.00 ± 5.00^{a}	14.00 ± 2.00^{b}	23.00 ± 2.00^{c}
Starch digestibility [%]	86.49 ± 0.18^{c}	84.31 ± 0.14^{b}	82.82 ± 0.30^{a}
Alfa-amylase inhibitor [kIU/100 mg dw]	14.40 ± 0.97^{b}	11.40 ± 0.89^{a}	14.70 ± 0.96^{b}
Amyloglucosidase inhibitor [kIU/100 mg dw]	0.28 ± 0.05^{b}	0.14 ± 0.03^{a}	0.17 ± 0.05^{ab}

Mean values with different letters in the row are statistically significantly different, with significance indicated as $p < 0.05$. "±" means standard deviation.

rich sprouts, because the lower availability is compensated by the higher content. Modification of buckwheat seeds with a probiotic yeast strain resulted in a higher total content of free sugars compared to sprouts and decreased the amount of total starch. The lower amount of starch in the probiotic-rich sprouts may be due to the use of the previous starch yeast as an energy material. It was noticed that the glycemic index and load increased in the germination process. However, the glycemic index values still appear around the low glycemic index.

Therefore, it is crucial to continue research using probiotic-rich sprouts, e.g. in human studies. The current trend of searching for new functional raw materials offers many opportunities. Improved nutritional quality can help to solve many of the problems where plant foods are the sole or main source of protein, and also plant fodder for livestock, which is then used as human food. However, an understanding of the basic metabolic regulation of, for example, amino acids is essential for such manipulation to be effective.

CRediT authorship contribution statement

Marta Molska: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Project administration. **Julita Reguła:** Supervision, Conceptualization, Methodology, Project administration, Formal analysis, Writing – review & editing. **Magdalena Zielińska-Dawidziak:** Conceptualization, Investigation, Writing – review & editing. **Aneta Tomczak:** Investigation, Writing – review & editing. **Michał Świeca:** Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Analysis of Phenolic Compounds in Buckwheat (*Fagopyrum esculentum* Moench) Sprouts Modified with Probiotic Yeast

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Abstract: Buckwheat sprouts are a source of various nutrients, e.g., antioxidant flavonoids, which have a positive effect on human health. This study analyzed the content of phenolic compounds and assessed their impact on the antioxidant and anti-inflammatory properties and dietary fiber in modified buckwheat sprouts. For this purpose, the buckwheat seeds were modified by adding *Saccharomyces cerevisiae* var. *boulardii*. The modified buckwheat sprouts showed a higher content of total phenol compounds (1526 μg/g d.w.) than the control sprouts (951 μg/g d.w.) and seeds (672 μg/g d.w.). As a consequence, a higher antioxidant activity and anti-inflammatory effect were noted. Probiotic-rich sprouts also had the highest content of total dietary fiber and its soluble fraction. A correlation between phenolic compounds and the antioxidant and anti-inflammatory effects, as well as dietary fiber, was shown. The interaction between dietary fiber and phenolic compounds affects the bioaccessibility, bioavailability, and bioactivity of phenolic compounds in food. The introduction of probiotic yeast into the sprouts had a positive effect on increasing their nutritional value, as well as their antioxidant and anti-inflammatory activity. As a consequence, the nutraceutical potential of the raw material changed, opening a new direction for the use of buckwheat sprouts, e.g., in industry.

Keywords: phenolic compounds; antioxidant activity; anti-inflammatory activity; dietary fiber; nutraceutical potential; germination; common buckwheat; *Saccharomyces boulardii*

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1. Introduction

Buckwheat belongs to the *Polygonaceae* family and is classified as a pseudocereal. Common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) are the main species of buckwheat consumed by humans [1,2].

Buckwheat (*Fagopyrum esculentum* Moench) is a crop mainly grown for its seeds [3]. There are various buckwheat-based foods on the market today, such as bread, pasta, tea, sprouts, and vinegar [4]. Sprouts are recognized as a unique dietary vegetable in Asia, Europe, and the United States. They are gaining more and more attention, as consumers require minimally processed, no additive, natural, nutritious, and wholesome food. It is worth paying attention to the germination process; for example, buckwheat sprouts' total phenolics and antioxidant capacity are greater than that of seeds [5].

Food of plant origin is receiving increasing interest, in terms of its protective effect against various diseases; in addition to its main role, that is, providing essential nutrients [6,7]. Buckwheat is a source of many antioxidants such as polyphenols, including six main flavonoids: rutin, orientin, vitexin, quercetin, isovitexin, and isoorientin [1,8]. Polyphenols are plant components that influence many chemical and biochemical reactions taking place in plant tissue. As components of plant raw materials used in nutrition, they influence sensory values and biological activity. Therefore, polyphenols are responsible for taste and color, as well as for antioxidant activity and health-promoting effects [9–11].

Abiotic and biotic factors affect the quality and quantity of crops. Quality can be defined as agronomic (e.g., fruit size, yield, and resistance to bacteria and fungi), organoleptic (e.g., color, firmness, and shape), and the nutrient and vitamin content. Stress from unfavorable stimuli can reduce yields. Abiotic factors include the soil composition, acidity, extreme salinity, high and low temperatures, drought, pollution, rain, wind, humidity, and ultraviolet radiation. On the other hand, biotic factors include various fungi, bacteria, and viruses that can cause many diseases [4,12,13].

Plants try to defend themselves and adapt to ecosystem conditions by releasing oxygen through photosynthesis, metabolic processes such as respiration, and the regulation of reactive oxygen species (ROS) [14]. Oxidative stress is described as an imbalance between the elimination of free radicals (oxidants) and production. The properties of antioxidants have been revealed, and they are considered protective against cancer and cardiovascular disease [15]. Buckwheat sprouts are rich in flavonoid compounds, thanks to which they are considered a health-promoting product with antioxidant and antihypertensive properties [1,16].

Germination is a process in which the primary, as well as secondary, active metabolites are increased. This is due to the fact that the seeds undergo a series of morphological and physiological changes. Active phytochemicals (e.g., phenols and flavonoids) gradually increase during the germination process. A higher level of polyphenolic compounds translates into the higher antioxidant activity of buckwheat sprouts. It is worth noting that different flavonoids may contribute differently to the overall antioxidant activity of different buckwheat species [17,18].

Flavonoids may be a promising molecule for solving chronic inflammatory processes. The results obtained in the publication by Ribeiro et al. 2015 showed promising effect of flavonoids in modulating the inflammatory process (namely those that represent a catechol group in the B-ring). This is because some flavonoids were able to simultaneously inhibit the production of pro-inflammatory prostaglandin E2 and pro-inflammatory cytokines, due to their ability to scavenge free radicals. In this way, they are excellent candidates for the prevention of diseases associated with increased production of free radicals, including cancer [14,19]. Buckwheat flavonoids were able to induce apoptosis in human leukemic cells HL-60 [20]. Ishii et al. [10] reported the anti-inflammatory effects of buckwheat sprouts on human colon cancer cells [21].

Buckwheat sprouts showed a strong anti-inflammatory effect by inhibiting inflammatory cytokines (e.g., IL-6), some inflammatory mediators (e.g., COX-2), and tumor necrosis factor-α (TNF-α). The anti-inflammatory properties of buckwheat sprouts are attributed to their rich content of phenolic compounds [4].

Taking into account the above information, it is necessary to develop effective techniques for the effective recovery/extraction and accurate determination of phenolic compounds. Phenolic compounds in plants are usually bound or free. The bound phenolic compounds are usually isolated by acid and alkali hydrolysis, followed by solvent extraction. Whereas, free phenolic compounds are usually extracted from plant samples using organic solvents (e.g., methanol, ethanol, and acetone) [22,23].

Probiotics are "live strains of strictly selected microorganisms which, when administered in adequate amounts, confer a health benefit on the host". They can affect food's organoleptic and microbiological quality, thanks to which they are used in traditional food products, e.g., fermented milk products. Moreover, probiotics can be used as part of products, which are their carriers. In recent years, e.g., chocolate and fruit drinks have been used as the probiotic carriers [24,25].

Another possibility of using probiotic microorganisms is to modify raw materials by adding them. Sprouts' metabolism can be additionally modified to obtain a more intensive growth or an increased concentration of bioactive ingredients. On the other hand, in the study by Świeca et al., 2019, the authors showed that legume sprouts enriched with *Saccharomyces cerevisiae* var. *boulardii* represented a new functional product characterized by increased health and nutritional properties [26,27]. At the same time, buckwheat is gaining

attention as a potential functional food. It is noted that both the raw material itself and products enriched with buckwheat are associated with many health benefits [28–30].

The germination process significantly increases the nutritional value by increasing the bioavailability of certain nutrients, e.g., vitamins [31]. Modifying the seeds by adding probiotics may also change the bioavailability of the ingredients, as well as their quantity. In the publication by Molska et al., in 2022, it was noticed that the protein availability was lower in the probiotic group. However, this was compensated for by the higher amount of protein in this group [32]. In subsequent studies, changes were noticed in the composition and amounts of individual sterols, stanols, and fatty acids [29,30]. Moreover, it has been also proven that co-culture of sprouts and bacteria affects the microbiological quality of the final product, slightly decreasing the total count of mesophilic bacteria. After 3 days of sprouting, a single edible portion of sprouts enriched with probiotic yeast contained an amount classifying them as a probiotic product (6.5 log/100 g f.m.) [29].

Hence, in this study, modified buckwheat sprouts were analyzed. Production of buckwheat sprouts was modified by introducing the probiotic yeast *Saccharomyces cerevisiae* var. *boulardii* during the soaking process. Therefore, the hypotheses assumed that such a modification would change the content of bioactive compounds (phenolics, dietary fiber) and affect the nutraceutical potential (antioxidant and anti-inflammatory properties) of the obtained buckwheat sprouts. The aim of the research was to analyze the content of phenolic compounds and to evaluate their influence on the antioxidant and anti-inflammatory properties, as well as the dietary fiber in modified buckwheat sprouts.

2. Results and Discussion

2.1. Phenolic Compounds

In recent years, many studies have been carried out on the composition of polyphenols in food, as well as their bioavailability and metabolism. This increased interest in polyphenols may be due to the results of epidemiological studies that have linked the consumption of diets rich in plant foods with a reduced risk of diseases related to oxidative stress [33,34]. Table 1 presents the phenolic compounds identified in *Fagopyrum esculentum* Moench buckwheat seeds and sprouts. Figure 1 shows a chromatogram of buckwheat polyphenols obtained using the UPLC-PDA-MS method.

Table 1. Qualitative–quantitative analysis of phenolic compounds in buckwheat forms.

	Compound	Rt	λ_{max}	(M-H) m/z		Sample		
		min	nm	MS	MS/MS	Seeds (G)	Control Sprouts (CS)	Probiotic-Rich Sprouts (PRS)
	Phenolic acids							
1	Caffeoyl-glucoside	2.19	288	341	179. 143	47.29 ± 0.41 ^{a}	53.23 ± 0.06 ^{a,b}	55.48 ± 0.52 ^{b}
2	Caffeoyl-rhamnopyranosyl-glucopyranosyl-glucopyranoside	2.47	289	649	487. 179	24.07 ± 0.54 ^{a}	27.80 ± 0.25 ^{a}	29.57 ± 0.92 ^{a}
3	Caffeoyl-rhamnopyranosyl-glucopyranosyl	3.50	288	487	179	79.80 ± 0.30 ^{a}	92.04 ± 0.05 ^{b}	118.23 ± 0.04 ^{c}
Flavan-3-ols								
1	Unknown catechin derivate	2.57	272	535	515. 267	73.52 ± 0.01 ^{c}	61.92 ± 0.23 ^{b}	61.89 ± 0.08 ^{a}
2	(Epi) afzelechin-(epi)-catechin	2.76	277	561	289. 245	9.47 ± 0.25 ^{a}	38.24 ± 0.89 ^{b}	56.80 ± 0.13 ^{c}
3	Catechin-glucoside	2.91	276	451	289	39.06 ± 0.43 ^{a}	62.08 ± 0.94 ^{b}	113.90 ± 0.71 ^{c}
4	Catechin-3-*O*-glucoside-6-*O*-rutinoside	3.09	278	719	451. 289	29.55 ± 0.07 ^{a}	32.60 ± 0.29 ^{b}	46.19 ± 0.92 ^{c}

Table 1. *Cont.*

	Compound	Rt	λ_{max}	(M-H) m/z		Sample		
		min	nm	MS	MS/MS	Seeds (G)	Control Sprouts (CS)	Probiotic-Rich Sprouts (PRS)
5	Caffeoyl-glucoside	3.31	288	341	179	2.31 ± 0.99 [a]	17.55 ± 0.5 [b]	33.51 ± 0.38 [c]
6	(+)Catechin	3.34	276	289	-	11.59 ± 0.11 [a]	27.85 ± 0.1 [b]	45.14 ± 0.75 [c]
7	Catechin-glucoside	3.40	279	451	289	18.35 ± 0.11 [a]	29.78 ± 0.2 [b]	42.66 ± 0.21 [c]
8	Epicatechin-(4-8)-epicatechin	3.61	277	577	289	107.36 ± 0.19 [a]	100.88 ± 0.05 [a]	115.94 ± 0.62 [b]
9	Epicatechin-(4-8)-epigallocatechin-gallate	3.70	279	729	577. 407. 289	15.79 ± 0.54 [a]	34.96 ± 0.76 [b]	57.89 ± 0.40 [c]
10	Epicatechin gallate dimethyl derivative	3.81	284	469	425. 137	3.45 ± 0.42 [a]	49.86 ± 0.48 [b]	63.37 ± 0.93 [c]
11	Epicatechin gallate	3.97	317	883 2 $[M-H]^-$	441. 289	27.17 ± 0.805 [a]	28.55 ± 0.45 [a]	70.88 ± 0.34 [b]
12	(-)Epicatechin	4.14	277	289	-	14.01 ± 0.74 [a]	39.60 ± 0.62 [b]	66.55 ± 0.07 [c]
13	Catechin trimer	4.27	279	865	577. 289	11.26 ± 0.01 [a]	19.80 ± 0.32 [b]	32.73 ± 0.71 [c]
14	Epicatechin gallate methyl derivative	4.44	292	455	441. 289	7.20 ± 0.86 [a]	113.27 ± 0.02 [b]	138.25 ± 0.98 [c]
15	Epicatechin trimer	4.62	279	865	577. 289	60.67 ± 0.18 [b]	22.61 ± 0.11 [a]	57.14 ± 0.3 [b]
16	Epiafzelechin-epicatechin-gallate dimethyl derivative	6.92	279	741	605. 469. 271	20.29 ± 0.22 [a]	10.75 ± 0.35 [a]	19.21 ± 0.98 [a]
17	Epiafzelechin-epicatechin-gallate methyl derivative	7.88	271	727	601. 407. 289	21.39 ± 0.2 [b]	5.68 ± 0.16 [a]	12.32 ± 0.62 [a]
Flavonols								
1	Orientin	4.67	269. 347	447	285	7.31 ± 0.43 [a]	3.44 ± 0.54 [a]	43.51 ± 0.42 [b]
2	Isorientin	4.87	270. 312	447	285	ND	6.42 ± 0.56 [a]	35.83 ± 0.25 [b]
3	Quercetin-3-*O*-rutinoside	5.24	255. 352	609	301	41.28 ± 0.88 [a]	52.48 ± 0.34 [b]	88.37 ± 0.42 [c]
4	Vitexin	5.35	269. 329	431	269	ND	20.09 ± 0.87 [a]	121.03 ± 0.58 [b]
	Total phenols compounds (μg/g d.w.)					672.14 ± 0.92 [a]	951.42 ± 1.82 [b]	1526.34 ± 3.33 [c]

Mean values with different letters in the row are statistically significantly different, with significance indicated as $p \leq 0.05$. "±" means standard deviation. ND—not detected.

Three phenolic acids (caffeoyl-glucoside, caffeoyl-rhamnopyranosyl-glucopyranosyl-glucopyranoside, and caffeoyl-rhamnopyranosyl-glucopyranosyl) were identified in both the seeds and the sprouts of buckwheat. Caffeoyl-rhamnopyranosyl-glucopyranosyl was the dominant phenolic acid in the grains and buckwheat sprouts; their amount in seeds was 79.80 ± 0.30 μg/g d.w., control sprouts 92.04 ± 0.05 μg/g d.w., and probiotic-rich sprouts 118.23 ± 0.04 μg/g d.w. The caffeoyl content was 47.29 ± 0.41 μg/g d.w. in seeds, 53.23 ± 0.06 μg/g d.w. in control sprouts, and 55.48 ± 0.52 μg/g d.w. in probiotic-rich sprouts.

Seventeen flavan-3-ols have also been identified in common buckwheat. There were statistically significant differences between the three identified samples in the case of twelve compounds (unknown catechin derivate, (epi) afzelechin-(epi)-catechin, catechin-glucoside, catechin-3-O-glucoside-6-O-rutinoside, caffeoyl-glucoside, (+)catechin, catechin-glucoside, epicatechin-(4-8)-epigallocatechin-gallate, epicatechin gallate dimethyl derivative (-)epicatechin, catechin trimer, and epicatechin gallate methyl derivative). In the next five, there were statistically significant differences between the control sprouts and the probiotic-rich sprouts: epicatechin-(4-8)-epicatechin, epicatechin gallate, epicatechin trimer,

epiafzelechin-epicatechin-gallate dimethyl derivative, and epiafzelechin-epicatechin-gallate methyl derivative.

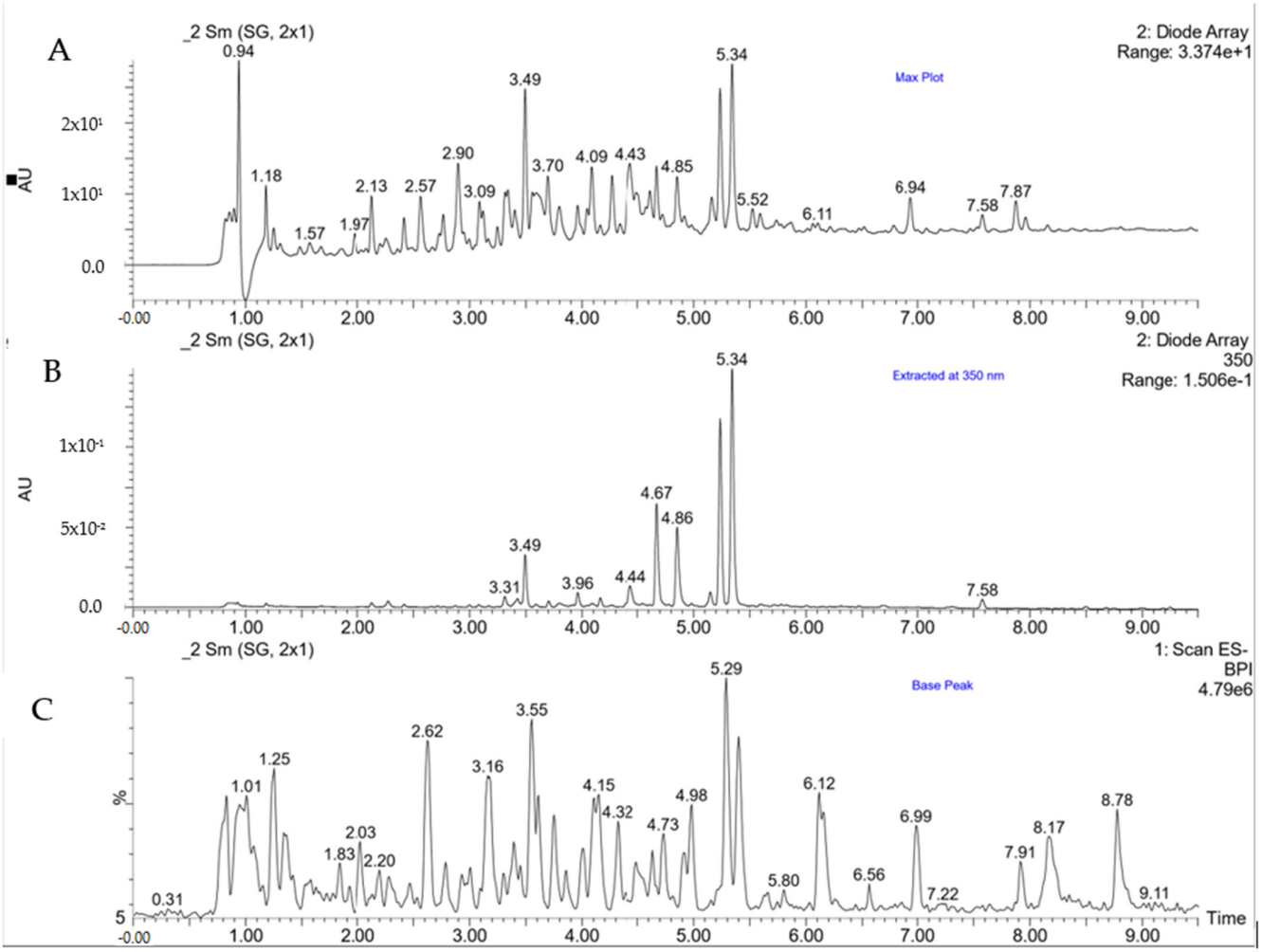


Figure 1. Chromatogram of buckwheat polyphenols obtained using UPLC-PDA-MS. (**A**)—Chromatogram UV-Vis recorded at max plot, (**B**)—chromatogram UV-VIS extracted at λ = 350 nm, (**C**)—base peak chromatogram recorded at full scan (ESI-MS).

Among all identified compounds, the modified buckwheat sprouts were characterized by the highest content of epicatechin gallate methyl derivative, i.e., 138.25 ± 0.98 μg/g d.w.; then, respectively, control sprouts (113.27 ± 0.02 μg/g d.w.) and seeds (7.20 ± 0.86 μg/g d.w.). This example shows that the germination process had a significant impact on the change in the content of bioactive compounds in the raw material. During the germination process, active phytochemicals such as phenols and flavonoids gradually increased in the buckwheat species [18].

It is noteworthy that the probiotic-rich sprouts also had the highest content of epicatechin-(4-8)-epicatechin and catechin-glucoside compared to the seeds and control sprouts. On the other hand, the lowest amount was found for the epiafzelechin-epicatechin-gallate methyl derivative, which was 12.32 ± 0.62 μg/g d.w. in the modified sprouts, 5.68 ± 0.16 μg/g d.w. in the control sprouts and 21.39 ± 0.2 μg/g d.w. in seeds. Comparing the content of flavan-3-ols in the control sprouts and sprouts rich in probiotics, it can be seen that the modification increased the amount of these bioactive compounds.

Regarding the flavanols, orientin, isoorientin, quercetin-3 O-rutinoside (rutin), and vitexin were identified. Orientin and vitexin were not identified in seeds. The flavanol with the highest amount in the raw material was vitexin, which increased by 100.94 μg/g d.w.

compared to the control sprouts. In the publication of Wiczkowski et al. 2014, the authors found that the main flavonols in buckwheat were orientin, isoorietin, and vitexin, as well as isovitexin. On the other hand, in Tartary buckwheat sprouts, these were detected in negligible amounts or not at all [35,36].

Buckwheat grains, as well as sprouts, are important sources of rutin, and the content depends on the growing conditions and type [37]. However, it has been noticed that *Fagopyrum esculentum* Moench has a several-times-lower rutin content than *Fagopyrum tataricum* L. Gaertn. [38]. Rutin lowers high blood pressure and has antioxidant and lipid peroxidative effects. It is worth noting that it also has a lipid-lowering effect, reducing the absorption of cholesterol from the diet. It causes a lower cholesterol level in the liver and plasma [37,39].

The level and potential bioavailability of pro-health properties are presented in Table 2 and Figure 2. The majority of phenolics belong to the flavonoids. In this section, we focused only on the quantitative analysis of this group of metabolites. The total content of flavonoids decreased in the following order: probiotic-rich sprouts > control sprouts > grains (statistically significant values). In the publication of Qin et al. 2010, the authors showed that the total content of flavonoids in buckwheat seeds was 0.67–2.25 mg/g d.w. [40]. It can be seen that, in this case, the amount of flavonoids was more than two-times higher in the modified sprouts than in the control sprouts. However, in the presented study, the total content of flavonoids was 14.1 ± 0.64 (mg QE per g d.w.). The value after digestion was 4.88 ± 1.18 (mg QE per g d.w.). A similar situation was seen in the case of the sprouts.

Table 2. Antioxidant activity and total flavonoid content in various forms of buckwheat.

	Before Digestion			After Digestion		
	Seeds (G)	**Control Sprouts (CS)**	**Probiotic-Rich Sprouts (PRS)**	**Seeds (G)**	**Control Sprouts (CS)**	**Probiotic-Rich Sprouts (PRS)**
Total flavonoids content (mg QE per g d.w.)	14.10 ± 0.64 ^{a}	20.88 ± 0.59 ^{b}	52.98 ± 0.77 ^{c}	4.88 ± 1.18 ^{a}	7.75 ± 0.78 ^{b}	15.75 ± 0.62 ^{c}
$ABTS^{+\bullet}$ (mg TE/1 g)	5.77 ± 0.83 ^{a}	12.63 ± 0.50 ^{b}	19.78 ± 1.37 ^{c}	8.82 ± 0.42 ^{a}	9.13 ± 0.54 ^{a}	11.05 ± 0.29 ^{b}
Metal chelating activity (mg EDTA/g d.w.)	4.08 ± 0.5 ^{a}	11.58 ± 0.34 ^{b}	11.70 ± 0.13 ^{b}	11.40 ± 1.23 ^{a}	102.15 ± 8.10 ^{b}	117.78 ± 4.95 ^{c}
Reducing power (mg TE/g d.w.)	11.24 ± 0.75 ^{a}	17.59 ± 0.84 ^{b}	31.15 ± 0.42 ^{c}	2.87 ± 0.05 ^{a}	6.50 ± 0.11 ^{b}	11.24 ± 0.34 ^{c}
COX-1 inhibitory activity (IU/g d.w.)	1664.18 ± 0.37 ^{a}	2119.97 ± 4.51 ^{b}	3037.15 ± 2.30 ^{c}	390.20 ± 0.39 ^{a}	682.00 ± 5.24 ^{b}	904.00 ± 4.92 ^{c}
COX-2 inhibitory activity (IU/g d.w.)	1215.42 ± 0.50 ^{a}	1562.75 ± 0.50 ^{b}	2431.24 ± 0.17 ^{c}	128.00 ± 0.50 ^{a}	213.62 ± 0.25 ^{b}	470.00 ± 0.25 ^{c}
LOX inhibitory activity (IU/g d.w.)	442.78 ± 5.91 ^{a}	740.80 ± 10.55 ^{b}	4721.56 ± 9.34 ^{c}	693.94 ± 12.91 ^{a}	773.69 ± 8.35 ^{b}	1250.00 ± 5.52 ^{c}

Mean values with different letters in the row are statistically significantly different, with significance indicated as $p \leq 0.05$. "±" means standard deviation.

It is worth noting that *Saccharomyces boulardii* influences the synthesis of active phytochemicals such as phenols, including isoflavones, and thereby increasing the antioxidant capacity of the products [41,42]. It is supposed that the increase in phenolics in co-culture may be due to different mechanisms. Previously it was proven that the components of yeast cell walls (chitin, chitosan) can act as effective elicitors promoting the de novo synthesis of pathogen-related compounds e.g., phenolics [43]. This strategy successfully increased the phenolic content and resulted from the pro-health properties of kidney bean [44] and wheat sprouts [45]. On the other hand, yeast effectively growing into the seed may cause a loosening of the seed structure, which promotes the release of bound phenolics from buckwheat cell walls [46].

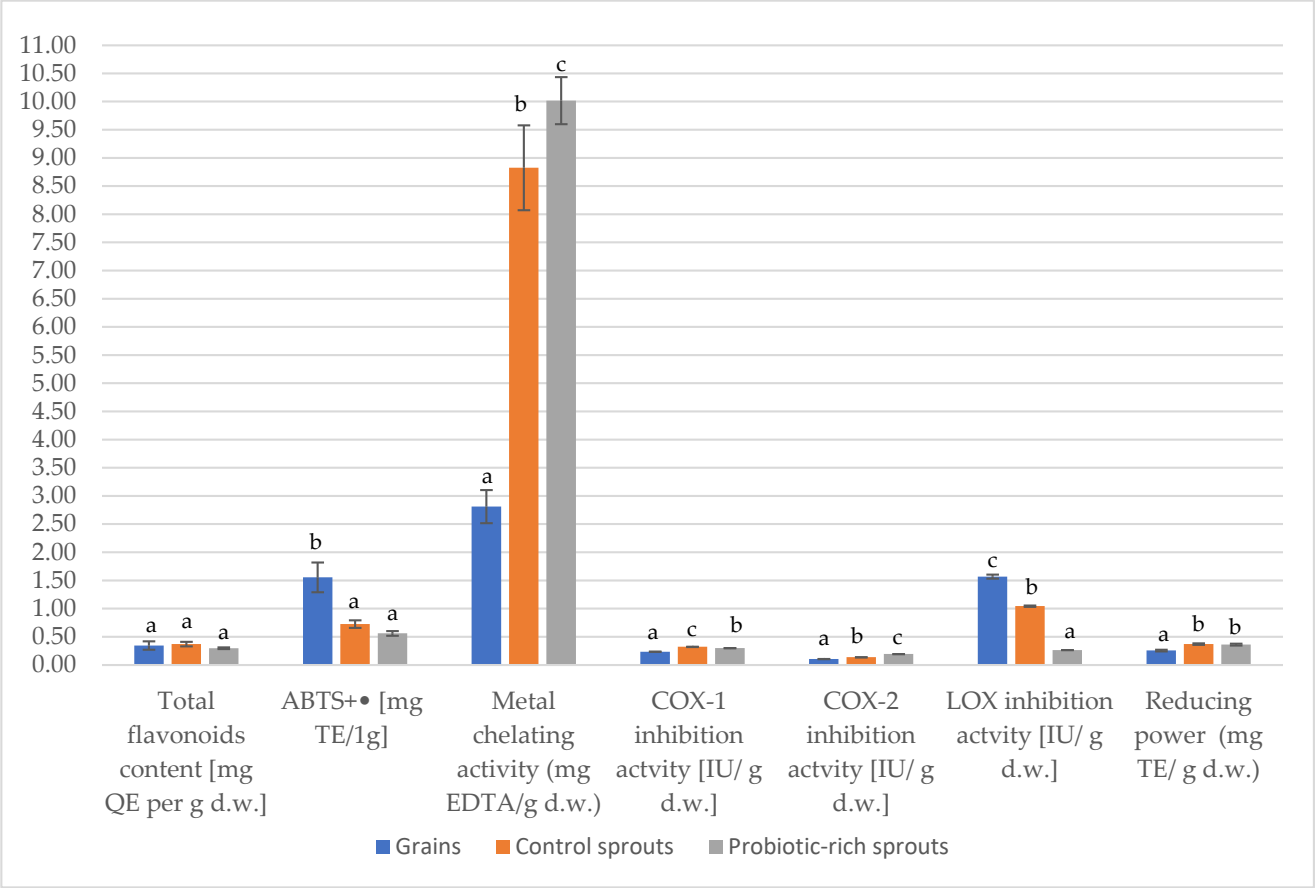


Figure 2. Comparison of the gastrointestinal digestibility index (IA). Mean values with different letters above the bars are statistically significantly different ($p \leq 0.05$). Statistical analysis for each presented parameter was carried out separately.

2.2. Antioxidant Activity

The content of flavonoids in probiotic-rich sprouts was significantly higher than in the other forms of buckwheat. Consequently, the modified sprouts showed the highest antioxidant potential, expressed as, inter alia, the ability to quench cationic radical $ABTS^{+\bullet}$. A similar relationship was noticed in the publication by Kędzierska-Matysek et al. 2021, in the case of buckwheat honey [47].

It is noted that, simultaneously with the increase in the total content of flavonoids in sprouts, the reduction power and the ability to scavenge free radicals increased proportionally. $ABTS^{+\bullet}$ values ranged from 5.77 to 19.78 mg TE/g (before digestion). Of which the most active were the modified sprouts. In contrast, the seeds (11.24 mg TE/g) had the lowest reduction power, while the modified sprouts (31.15 mg TE/g) were the highest. The "bioavailability" of the reduction force decreased in the following direction: control sprouts > probiotic-rich sprouts > seeds.

The presence of transition metal ions plays a significant role in oxidative processes leading to the formation of superoxide anion radicals and singlet oxygen during the Fenton reaction and during changes during the storage and thermal processing of food. In addition, metal ions are catalysts for the decomposition of lipid hydroperoxides. These processes can be delayed by the chelation and deactivation of iron ions present in the system [48]. Flavonoids can indirectly chelate transition metal ions, i.e., copper and iron, which prevents the formation of reactive hydroxyl radicals in cells. They also contribute to the stabilization of cell membranes [49]. The chelating capacity of metals changed similarly to the indices mentioned above. However, what distinguishes this was the increase in the activity in the digestive process. After digestion, the values were higher: 35.39 ± 1.23 mg EDTA/g d.w.

in seeds, 102.15 ± 8.10 mg EDTA/g d.w. in control sprouts, and 117.78 ± 4.95 mg EDTA/g d.w. in modified sprouts. The bioavailability of the chelating capacity was highest in the probiotic-rich sprouts (Figure 2).

The strong metal chelating activity of probiotic-rich sprouts could have been due to the higher content of quercetin compared to the seeds. It has more structural features than complex metal ions. The therapeutic benefits of chelators are noted in metal catalyzed chronic diseases induced by oxidative stress, e.g., in cardiovascular diseases [50,51]. The strong chelating activity of buckwheat metals may be due to the higher content of, not only quercitin, but also rutin, as they have more of the structural features of complexing metal ions [4].

2.3. Anti-Inflammatory Activity

The inhibition value of cycloxygenase 1 (COX-1) and 2 (COX-2) decreased analogously to the flavonoids, from probiotic-rich sprouts to seeds. The bioavailability of COX-1 was lowest in seeds and highest in control sprouts (Figure 2). In the case of COX-2, the bioavailability was lower, as follows: 0.11 in seeds, 0.14 in control sprouts, and 0.19 in probiotic-rich sprouts. COX-2 overexpression plays a key role in several inflammatory diseases. In contrast, inhibition of COX-2 expression is one of the therapeutic targets for inflammation [52]. The value of lipoxygenase inhibition in the modified vines was six times higher than in the probiotic-rich sprouts. The interest in lipoxygenases on the part of food technologists results from their ability to produce free radicals and peroxides, which are involved in the oxidation of vitamins, dyes, phenolic compounds, and proteins [53]. The anti-inflammatory properties of buckwheat sprouts have been attributed to their high content of phenolic compounds, especially rutin and possibly quercetin [4,54]. It has been reported that buckwheat extracts have a strong inhibitory effect on, e.g., inflammatory mediators in lipopolysaccharide-stimulated RAW 264.7-induced and peritoneal macrophages [55]. In the publication by Karki et al., 2013, buckwheat extract and rutin significantly inhibited the expression of COX-2, which may suggest their beneficial role in the treatment of inflammation [4]. Almuhayawi et al. (2021) reported that buckwheat extracts directly inhibited the activity of COX-2 and lipoxygenase [56].

Thus, *Fagopyrum esculentum* Moench and the flavonoids it contains are potential natural therapeutic agents against inflammation [23].

2.4. Saccharomyces cerevisiae var. boulardii

Digestion increases the antioxidant capacity of cereal and pseudocereal products. It is worth noting that digestibility is, therefore, considered to be an essential factor in enhancing the antioxidant capacity of foods such as whole grains. In the study by Kim et al., 2013, it was noticed that consumption of buckwheat simultaneously improved antioxidant processes and peroxidation, reducing the damage caused by oxidative stress [4,57,58].

There is a growing interest in adding probiotic cultures to foods. The purpose of this is to develop foods with health-promoting properties, and it is worth emphasizing that functional foods use many lactobacilli. Despite the presence of yeast in many dairy products, the active use of yeast as a probiotic has been limited. *Saccharomyces cerevisiae* var. *boulardii* is classified as a yeast species with probiotic properties. It is used prophylactically and therapeutically in the treatment of various diarrheal diseases [59,60].

Saccharomyces cerevisiae var. *boulardii* affects the synthesis of active phytochemicals, such as phenols, and including isoflavones [41,42]. Thus, increasing the antioxidant capacity of the products. Furthermore, when it comes to improving nutritional value, this yeast is known to catalyze the breakdown of phytate in the diet. This results in a significant improvement in the bioavailability of essential minerals [41,61,62]. The antioxidant capacity of this strain was established by Datta et al., in 2017. They compared *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae* var. *boulardii* (NCYC-3264), paying attention to their antioxidant capacity, response to various stress conditions, as well as the production of important secondary metabolites. *S. boulardii* showed a better tolerance to stress but showed no

significant differences in growth patterns compared to *S. cerevisiae*. Additionally, it is worth noting that *Saccharomyces cerevisiae* var. *boulardii* produced a comparatively (6 to 10 times) greater antioxidant potential, as well as 20- and 70-times the total level of flavonoids and phenols in the extracellular fraction [63]. In the publication of Fernández-Pacheco et al., 2021, twenty strains of yeast isolated from various food ecosystems, the probiotic characteristics of which had previously been studied, were presented. On the other hand, in the publication mentioned above, the yeasts were examined, among other ways, in terms of their enzymatic and antioxidant activity. All the tested yeast strains showed antioxidant activity by scavenging free radicals from the medium. It is worth mentioning that they were catalase positive, which means that the yeast has an enzyme defense system that is capable of converting reactive oxygen species from hydrogen peroxide into H_2O and O_2 [64].

2.5. Dietary Fiber

Figure 3 shows the content of total, soluble, and insoluble fiber in various forms of buckwheat. In the examined seeds and buckwheat sprouts, different amounts of dietary fiber were observed. It was found that probiotic-rich sprouts had the highest content of total dietary fiber (16.11%), while the lowest content was found in seeds (11.37%). The dominant dietary fiber fraction in probiotic-rich sprouts was soluble dietary fiber. Taking into account the level of soluble dietary fiber, the various forms of buckwheat studied can be organized as follows: probiotic-rich sprouts > control sprouts > grains. In the case of the insoluble dietary fiber fraction, probiotic rich sprouts were characterized by a statistically lower amount compared to the control sprouts and seeds.

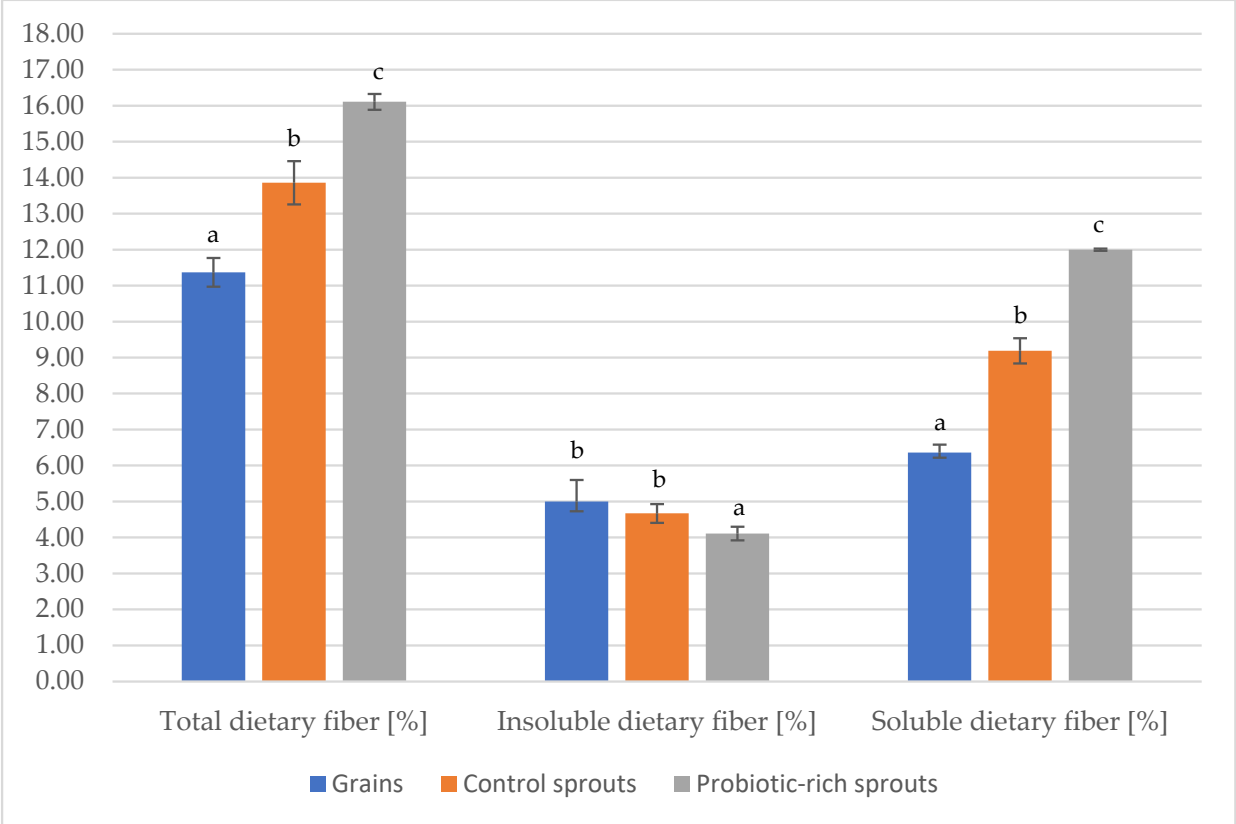


Figure 3. The content of total, soluble, and insoluble fiber in various forms of buckwheat. Mean values with different letters above the bars are statistically significantly different ($p \leq 0.05$). Statistical analysis for each presented dietary fiber fraction was performed separately.

The publication of Górecka et al., 2009, showed that buckwheat seeds have a higher content of insoluble than soluble fiber [65]. Interestingly, this is the opposite result to that obtained in the present work.

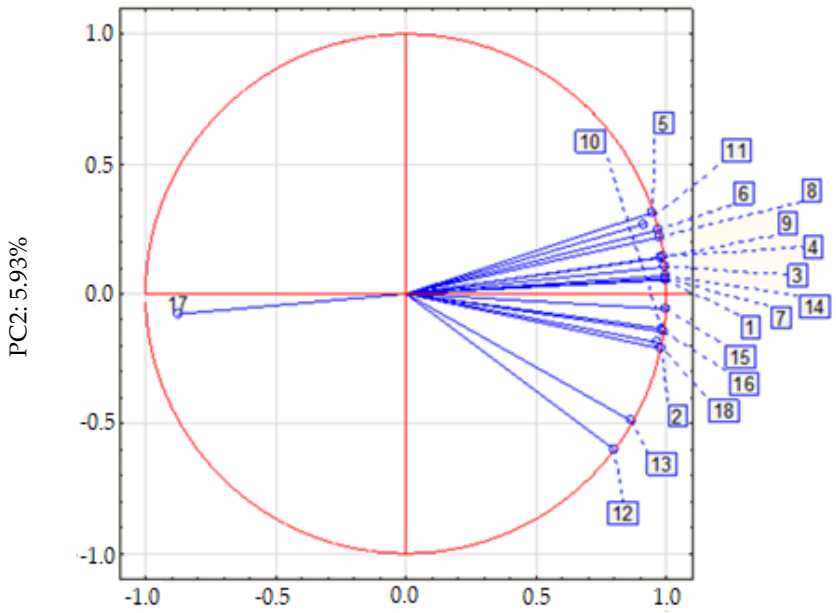
An increase in the dietary fiber in the probiotic-rich sprouts may be explained by the utilization of storage materials in growing sprouts and probiotic yeast. This caused an increase in dietary fiber in the dry biomass of the sprouts. This confirmed the results obtained in the publication by Molska et al., 2022, where the amount of resistant starch was higher in modified sprouts than in control sprouts [32]. The general behavior of resistant starch is physiologically similar to that of fermenting, soluble fiber [66]. On the other hand, β-glucan belongs to the soluble fraction of dietary fiber. Buckwheat may contain about 20 g/100 g DM of β-glucan. Moreover, yeast is also a source of this compound. The yeast cell wall (genus *Saccharomyces*) consists, inter alia, of β-glucans and mannoprotein [67,68].

The interaction between phenolic compounds and dietary fiber components affects the bioavailability, bioaccessibility, and bioactivities of phenolic compounds in foods. Studies by other authors have shown the nature of the interaction between different groups of phenolic compounds and the basic components of the cell wall matrix (e.g., polysaccharide groups in dietary fiber). Using pectin and cellulose as cell wall models, it was shown that phenolic acids and anthocyanins interact with both polysaccharides [69,70].

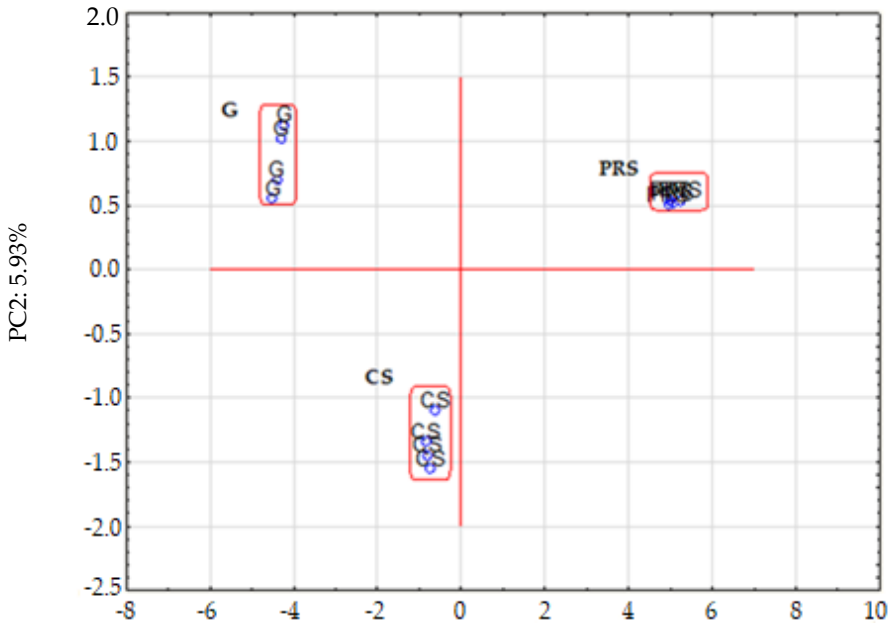
2.6. Principal Component Analysis

For a better understanding of the interactions between the analyzed values, we conducted a principal components analysis (PCA), Figure 4a,b. The dependencies of the variables are presented in Figure 4a. The created model (a system of 2 principal components) explained 97.48% of the overall variability. The interdependence of the vectors (Figure 4a) suggested that the content of total flavonoids before and after digestion, as well as the total content of phenols, showed a negative correlation with the insoluble fiber fraction. On the other hand, the total phenolic content and the total flavonoid content before and after digestion correlated positively with each other, as well as with the antioxidant indexes. The graph of the scattering of objects in the space defined by the first two main components (Figure 4b) also provides interesting information. There are three relatively compact clusters of points, depicting individual groups. Among which, the probiotic-rich group forms the strictest cluster. A Spearman correlation analysis also confirmed the correlations shown in the PCA analysis (Table 3).

Holasova et al. (2002) noted a strong correlation between the total phenol content and the antioxidant capacity of buckwheat seeds. They indicated that the total phenolics content probably indicates the antioxidant capacity [71]. However, it is worth noting that Hung and Morita (2008) found that the free phenolic compounds had a greater share in the scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl radicals, as well as the total antioxidant capacity of the buckwheat fraction, than the bound phenolic compounds [72].



(**a**)



(**b**)

Figure 4. Principal components analysis (PCA) for the tested parameters (projection of variables onto the plane of factors PC1 × PC2 (**a**), projection of cases onto the plane of factors (**b**)). Abbreviations: 1. COX-1 before digestion, 2. COX-1 after digestion, 3. COX-2 before digestion, 4. COX-2 after digestion, 5. LOX before digestion, 6. LOX after digestion, 7. Total phenol compounds, 8. Total flavonoids content before digestion, 9. Total flavonoids content after digestion, 10. $ABTS^{+\bullet}$ before digestion, 11. $ABTS^{+\bullet}$ after digestion, 12. Metal chelating activity before digestion, 13. Metal chelating activity after digestion, 14. Reducing power before digestion, 15. Reducing power after digestion, 16. Soluble fiber, 17. Insoluble fiber, 18. Total dietary fiber.

Table 3. Correlation between the bioactive compounds and the antioxidant and anti-inflammatory activities.

		Total Flavonoids Content (mg QE per g d.w.)		Total Phenols Compounds (µg/g DM)
		Before Digestion	**After Digestion**	
$ABTS^{+\bullet}$ (mg TE/1 g)	Before digestion	R = 0.872155 $p < 0.00$	R = 0.915499 $p < 0.00$	R = 0.947758 $p < 0.00$
	After digestion	R = 0.770579 $p < 0.00$	R = 0,721835 $p < 0.00$	R = 0.784862 $p < 0.00$
Metal chelating activity (mg EDTA/g d.w.)	Before digestion	R = 0.819616 $p < 0.00$	R = 0.713033 $p < 0.00$	R = 0.814480 $p < 0.00$
	After digestion	R = 0.853147 $p < 0.00$	R = 0,882262 $p < 0.00$	R = 0.946100 $p < 0.00$
Reducing power (mg TE/g d.w.)	Before digestion	R = 0.905269 $p < 0.00$	R = 0.945328 $p < 0.00$	R = 0.949425 $p < 0.00$
	After digestion	R = 0.888112 $p < 0.00$	R = 0.938502 $p < 0.00$	R = 0.946100 $p < 0.00$
COX-1 inhibitory activity (IU/g d.w.)	Before digestion	R = 0.882663 $p < 0.00$	R = 0.924302 $p < 0.00$	R = 0.947758 $p < 0.00$
	After digestion	R = 0.892807 $p < 0.00$	R = 0.909894 $p < 0.00$	R = 0.951101 $p < 0.00$
COX-2 inhibitory activity (IU/g d.w.)	Before digestion	R = 0.950583 $p < 0.00$	R = 0.873902 $p < 0.00$	R = 0.956183 $p < 0.00$
	After digestion	R = 0.888213 $p < 0.00$	R = 0.923267 $p < 0.00$	R = 0.961347 $p < 0.00$
LOX inhibitory activity (IU/g d.w.)	Before digestion	R = 0.937063 $p < 0.00$	R = 0.864687 $p < 0.00$	R = 0.946100 $p < 0.00$
	After digestion	R = 0.931700 $p < 0.00$	R = 0.832752 $p < 0.00$	R = 0.947758 $p < 0.00$
Total dietary fiber (%)		R = 0.904644 $p < 0.00$	R = 0,888112 $p < 0.00$	R = 0.956183 $p < 0.00$
Insoluble dietary fiber (%)		R = −0.791564 $p < 0.00$	R = −0.838378 $p < 0.00$	R = −0.836660 $p < 0.00$
Soluble dietary fiber (%)		R = 0.904644 $p < 0.00$	R = 0.888112 $p < 0.00$	R = 0.956183 $p < 0.00$

Statistically significantly different, with significance indicated as $p < 0.05$.

3. Materials and Methods

All chemicals used in the analyzes were purchased from Sigma Aldrich, Poznań, Poland, unless otherwise stated.

3.1. Buckwheat Sprouting

Fagopyrum esculentum Moench seeds were purchased from PNOS S.A. in Ożarów Mazowiecki, Poland. Buckwheat seeds were disinfected in 1% (*v*/*v*) sodium hypochloride (Sigma-Aldrich. USA) for 10 min. Afterwards, grains were washed with distilled water to reach neutral pH and soaked in distilled water (CS) or in *Saccharomyces cerevisiae* var. *boulardii* water suspension (1×10^7 CFU per 1 g of seeds; PRS) for 4 h. Germination was performed in a growth chamber (SANYO MLR-350H, Japan) on plates lined with absorbent paper for 3 days. Seedlings were sprayed with Milli-Q water daily. Germinated seeds (sprouts) were manually collected and rinsed with distilled water. Then, they were freeze-dried, milled, and frozen [29].

3.2. Phenolic Content

3.2.1. Extraction Procedure

Solid: Solvent Extraction

Lyophilized samples of seeds and sprouts (100 mg) were mixed with 5 mL of 50% methanol. Samples were sonicated at room temperature (25 ± 1 °C) (3 intervals of 30 s; 42 kHz, 135 W; Branson Ultrasonic Corporation, Brookfield, WI, USA) and extracted for 30 min in a labor shaker (MS3 Basic, Ika, Wilmington, DE, USA, 150 rpm). Then the samples were centrifuged (20 min 6800× g). The resulting supernatants were kept at −20 °C prior to analysis as chemical extracts (CE).

In Vitro Digestion

In vitro digestion was performed as described by Minekus et al., with some modifications [73,74]. After digestion, the samples were centrifuged (15 min 6900× g) and the supernatants were mixed with an equal volume of methanol to stop digestion. The potentially bioavailable fractions were frozen and kept at −20 °C.

3.2.2. Qualitative–Quantitative Analysis of Phenolic Compounds

The extracts for solid:solvent extraction were suspended in water (10 mL) and passed through a C18 Sep-Pak cartridge (360 mg, 55–105 µm) (Waters Associates, Milford, MA, USA) preconditioned with water. First, the cartridge was washed with water (10 mL), to remove sugars. Then, MeOH (10 mL) was used to elute the phenolic compounds. This fraction was evaporated to dryness; re-dissolved in 50% MeOH for analysis. General phenolic profiles and structural information were collected using reverse phase ultra-efficiency liquid chromatography (UPLC)-PDA-MS/MS Waters ACQUITY (Waters. Milford, MA, USA). This consisted of a binary solvent manager, sample manager, photodiode array detector (PDA), and triple quadrupole detector (TQD) operating with negative electrospray ionization.

Ion source parameters were as follows: cone voltage 35 V, capillary voltage 3 kV, extractor 3 V, RF lens 100 mV, source temperature 120 °C, desolvation temperature 350 °C, desolvation gas flow 800 L/h, cone gas flow 100 L/h, and the collision gas flow 300 µL/min. Collision cell parameters: collision energy 22 eV and input −2, output 0.5. The parameters of quadrupole 1 were set to achieve the maximum mass resolution. The LM and HM resolutions were set to 15 and the ion energy to 0.8. The collision cell parameters for the MS/MS experiments were as follows: gas collision pressure (argon), 1.5 × 10^{-3} mbar, and collision energy 15 or 30 eV. Acquisition in the MS scan mode and the product ion scan were performed in the centroid mode monitoring mode from 100 to 1200 m/z. Phenolic acids were separated on an Acquity BEH column with a diameter of 100 mm × 2.1 mm, 1.7 µm (Waters). A linear gradient of 8.5 min was applied from 80 to 100% solvent B (40% acetonitrile containing 0.1% formic acid) in solvent A (water containing 0.1% formic acid) at 0.35 mL/min. Results are expressed in µg per g dry weight (d.w.) as the equivalent of kaempferol 3-O-glucoside [75]. The method was validated for parameters such as the linearity, accuracy (relative error, RE), limit of detection (LOD), limit of quantification (LOQ), and precision (relative standard deviation, RSD). Stock standard solutions of the polyphenols were prepared with methanol. Six calibrators of each standard were prepared by dilution of stock solutions, and the calibration curve was generated by plotting the peak area ratio of the polyphenol versus the nominal concentration. A regression equation was obtained using weighted (1/c2) least-squares linear regression. The LOD was determined as a signal-to-noise ratio (S/N) of 3:1, and the LOQ was determined as a S/N of >10. An acceptable RE within ±20% and an RSD not exceeding 20% should be obtained.

3.2.3. Quantitative Analysis of Total Flavonoids Content (TFC)

The total content of flavonoids was determined according to the method described by Haile and Kang [76]. A 1 mL aliquot of the test solution was mixed with 0.3 mL of $NaNO_2$ (5% w/v). After 5 min, 0.5 mL of $AlCl_3$ (2% w/v) was added. Standard solutions

of flavonoids with a concentration of 100 µM were used. The sample was mixed and neutralized with 0.5 mL of 1 M NaOH solution 6 min later. The resulting mixture was allowed to stand for 20 min at room temperature. The absorbance at 510 nm was then measured. Total flavonoid content was calculated as quercetin equivalent (QE) in mg/g dry weight (d.w.).

3.3. Pro-Health Properties

3.3.1. Antiradical Activity ($ABTS^{+\bullet}$)

Experiments were performed using an ABTS bleaching test [77]. The radical cation ABTS ($ABTS^{+\bullet}$) was prepared by reacting a 7mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration). Leaving the mixture in the dark at room temperature for at least 6 h before use. The $ABTS^{+\bullet}$ solution was diluted to an absorbance of 0.7 ± 0.05 at 734 nm (Lambda 40 UV-Vis spectrophotometer, Perkin Elmer Inc. Waltham, MA, USA). Then, 50 µL of the extract obtained after in vitro digestion was added to 1.45 mL of the $ABTS^{+\bullet}$ solution. The absorbance was then measured at a final time of 15 min. The ability of the extracts to quench the ABTS free radical was calculated as a Trolox equivalents (TE) in mg per g of dry weight (d.w.).

3.3.2. Reducing Power (RP)

The reduction power was determined using the method developed by Pulido, Bravo, and Saura-Calixto 2000 [78]. The activities were expressed as Trolox equivalents in mg/g dry weight (d.w.).

3.3.3. Metal Chelating Activity (CHP)

Chelating power was determined using the method of Guo et al., 2001 [79]. Chelating power was expressed as EDTA equivalent in mg per g of dry weight (d.w.).

3.3.4. Ability to Inhibit the Activity of Cyclooxygenases (COX-1 and COX-2)

The ability of the extracts obtained to inhibit cyclooxygenase-1 and cyclooxygenase-2, after chemical extraction and digestion in vitro, was determined using a COX colorimetric inhibitor screening assay kit (Cayman Chemical, No. 701050). The results were expressed in inhibitory units (UIs) per g d.w. One IU was defined as inhibition of 1U of enzyme activity in 1 min.

3.3.5. Ability to Inhibit the Activity of Lipoxygenase (LOX)

A lipoxygenase inhibitory (LOXI) assay was carried out using linoleic acid as a substrate with the method described by Szymanowska et al. [80] adapted to a microplate reader. One unit of LOX activity was defined as an increase in absorbance of 0.001 per minute at 234 nm (equivalent to the oxidation of 0.12 µmole of linoleic acid). The results were expressed in inhibitory units (UIs) per g d.w. One IU was defined as inhibition of 1U of enzyme activity in 1 min.

3.4. Theoretical Approaches

The following factors were identified to better understand the potential bioaccesibility of biologically active extracts [75].

The gastrointestinal digestibility index (IA), which is an indicator of the bioaccesibility of antioxidants released in the digestive tract, was calculated as follows:

$$IA = A_{GDI}/A_{ce}$$

where A_{ce} is the activity of chemical extract (CE), and A_{GDI} is the activity of the extracts after simulated gastrointestinal digestion (GDI).

3.5. Dietary Fiber

Total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) contents were measured using a Megazyme total dietary fiber analysis kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The sum of IDF and SDF was the total dietary fiber (TDF). The content is expressed as a percentage.

3.6. Statistical Analysis

All experimental results were mean ± S.D. of three independent experiments (n = 9). One-way analysis of variance (ANOVA) and Turkey's post-hoc test were used to compare groups (seeds, as well as control and elicited sprouts) (STATISTICA 6, StatSoft, Inc., Tulsa, USA). Differences were considered significant at $p \leq 0.05$. The correlation between tested parameters was determined using principal component analysis (PCA). The Spearman correlation coefficient (R) and p-value were used to show correlations and their significance. Differences of $p < 0.05$ were considered significant.

4. Conclusions

The germination process changed the content of individual phenolic acids, as well as the parameters of the antioxidant activity in sprouts. When comparing the control and modified sprouts, a significant change was noticed in most phenolic acids. As a consequence, a higher antioxidant activity was noted. An association with anti-inflammatory activity and dietary fiber was also found. This is also indicated by the PCA analysis, which showed a positive correlation between total phenolic content and the total flavonoid content before and after digestion, correlating positively with each other, as well as with the antioxidant indexes. Modified buckwheat sprouts were characterized by the highest content of epicatechin gallate methyl derivative among the marked phenolic compounds.

Referring to the hypotheses in the work, the following points can be made:

1. A change in the amount of bioactive compounds was noticed in the modified buckwheat sprouts compared to the control sprouts;
2. the nutraceutical potential of the raw material was changed.

The presented research gives a new direction for the use of this raw material; common buckwheat sprouts. This information may be helpful for the food industry, aiming at producing buckwheat-based products with better nutritional and health properties.

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RESEARCH

Adding Modified Buckwheat Sprouts to an Atherogenic Diet — the Effect on Selected Nutritional Parameters in Rats

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Abstract

The germinated seeds of many plants are a natural source of substances that can be used to supplement food and increase its functionality. The seeds' metabolism may be modified during germination to produce specific health-promoting compounds. *Fagopyrum esculentum* Moench is a rich source of nutrients. Buckwheat seeds modified during germination may be helpful as an additive to new functional food products with anti-atherogenic properties. However, their effect and safety should be assessed in *in vivo* studies. The aim of the study was to evaluate the effect that adding modified buckwheat sprouts (*Fagopyrum esculentum* Moench) to an atherogenic (high-fat) diet has on the morphology and digestibility parameters of rats. Buckwheat seeds were modified by adding the probiotic strain of the yeast *Saccharomyces cerevisiae* var. *boulardii*. The study was carried out on 32 Wistar rats, and digestibility and blood counts were assessed during the experiment. There was no evidence of an adverse effect on the animals' weight gain and nutritional efficiency. However, the influence of diets with freeze-dried buckwheat sprouts on digestibility and morphological parameters was noticed. Fat digestibility registered a statistically significant decrease in the groups fed a high-fat diet with the addition of sprouts. The study shows a new direction in the use of buckwheat sprouts.

Keywords Apparent digestibility · Morphological analysis · *Fagopyrum esculentum* Moench · *Saccharomyces cerevisiae* var. *boulardii* · Pseudocereals

Abbreviations

AIN-93M	Standard diet
AIN-R	A group of rats fed the AIN-93M diet
BASO	Basophils
EOS	Eosinophils
HCT	Hematocrit
HFD	High-fat diet
HFD-R	The group fed the HFD diet
HFDCS	Modified AIN-93M diet by adding lard and buckwheat sprouts lyophilisate
HFDCS-R	A group of rats fed the HFDCS diet
HFDPRS	Modified AIN-93M diet by adding lard and modified buckwheat sprouts lyophilisate
HFDPRS-R	A group of rats fed the HFDPRS diet
HGB	Hemoglobin
LYM	Lymphocytes
MCH	Mean blood hemoglobin concentration
MCHC	Mean blood hemoglobin
MCV	Mean red blood cell volume
MONO	Monocytes
NEU	Neutrophils
PLT	Thrombocytes
RBC	Red blood cells
WBC	White blood cells

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Introduction

Buckwheat is a crucial pseudocereal produced in many countries, such as Japan, China, Russia, and European countries. *Fagopyrum esculentum* Moench, or common buckwheat, is a good source of pro-health ingredients, especially protein, bioactive compounds such as flavonoids, flavones,

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phenolic acids, tannins, phytosterols and phagopyrins, which can be found in the grain and husk of buckwheat [1]. It is usually used in grains and ground form, which is processed into other food products, such as pasta and bread. Furthermore, sprouting is an integral part of the cultivation process, resulting in sprouts with a nutritional value different from the seeds mentioned above [1, 2].

Sprouting is a method of improving a product's nutritional value and functionality. During this process, several changes occur in the raw material. For example, Yiming et al. [1] showed that the content of total flavonoids increased during the sprouting process. However, other studies have shown that fats, proteins, and starch are broken down into compounds that are a source of energy and substrates from which newly synthesized compounds are formed [1, 3–5].

The search for new functional products sets the trend for the industry and creates the need to check and confirm their pro-health effects. One of the products that can be used as an element of functional food are pseudocereals. Due to their excellent nutritional value, they have been referred to as "grains of the twenty-first century". They are rich in protein with a high biological value, starch, trace elements or a group of bioactive ingredients (saponins, phenolic compounds, phytosterols, phytoecdysteroids, polysaccharides, betalains and bioactive proteins and peptides). They also have a well-balanced amino acid composition [6–8].

Buckwheat protein was found to be poorly digestible, despite its high biological value. The poor digestibility and thus availability of buckwheat proteins is mainly caused by the high level of protease inhibitors (substances that inhibit the activity of proteolytic enzymes) and tannins (vegetable tannins, organic chemical compounds). A special feature of buckwheat kernels is the specific combination of phenolic compounds with proteins, which may reduce their enzymatic availability. One of the methods of improving digestibility may be the partial reduction of tannins, flavonoids or phenolic acids due to roasting and thermal deactivation of trypsin inhibitors [8–13].

Digestibility is essential in interpreting the quality of the nutrients the body digests. In practice, this is the difference between the amount of nutrient ingested and the amount produced in the stools. Food processing alters molecular, supramolecular structures, allowing digestive enzymes to access nutrients more easily. Consequently, it should improve the digestibility of the food. Germination or technological changes of grains create opportunities to obtain new sources of functional food rich in health-promoting substances. The nutritional composition of a sprout depends on factors such as the type of sprout and the germination conditions. Many biochemical changes occur in the germination process, thus affecting the nutritional value of the raw material. During this process, a large number of hydrolytic enzymes are synthesized or released, which degrade anti-nutritional factors or hydrolyze what might be termed biopolymers, *e.g*., proteins [5, 10, 14, 15].

On the other hand, adding sprouts to a product, for example, may change its nutritional value. Alvarez-Jubete et al. [16] observed that the baking process had a negative effect on the antioxidant properties of bread. Bread with the addition of buckwheat sprouts had a much higher total phenol content as well as a higher antioxidant capacity than the wheat bread used as a control [16]. In another study, adzuki bean sprouts and lentils were enriched and used as carriers for *Saccharomyces cerevisiae* var. *boulardii*. The authors found that the resulting sprouts enriched with *S. cerevisiae* var. *boulardii* were a new functional food product [17].

The yeast *Saccharomyces cerevisiae* is a very good model system for studying the function-gene relationship, *e.g*., in fatty acid metabolism, as Trotter [18] reports. Fatty acids are broken down by yeast only in peroxisomes. In contrast, genes encoding core as well as helper peroxisomal β-oxidation enzymes have recently been identified. The *de novo* fatty acid profile of yeast is characterized by a majority of saturated and monoenoic acids (containing 16 and 18 carbon atoms). However, approximately 1-2% of total fatty acids synthesized are fatty acids containing 20–30 carbon atoms [18, 19]. Modifying seeds by adding a probiotic yeast strain had a beneficial effect on the change in fatty acid composition (reduction in saturated fatty acids in favor of polyenic fatty acids) [20, 21]. It can also change the bioavailability of ingredients, as well as their quantity, *e.g*., protein [22].

Taking into account the above information, the following hypotheses were put forward: 1/ the modification of sprouts affects the digestibility of selected macronutrients and dry matter; 2/ a diet with probiotic-rich sprouts affects the nutritional parameters of the rats fed with it.

The aim of the study was to evaluate the effect of adding modified buckwheat sprouts (*Fagopyrum esculentum* Moench) to an atherogenic (high-fat) diet on the morphology and digestibility parameters of rats. Buckwheat seeds have been modified by adding the probiotic strain of the yeast *Saccharomyces cerevisiae* var. *boulardii*.

Materials and Methods

The material and methods section is presented as supplementary material 1.

Results and Discussion

Table 1S (in Supplementary Material 1) shows the energy and nutritional value of the dry matter in the experimental diets. The AIN-93M diet had the highest energy value (2.28 MJ/100 g), and the high-fat diet (1.85 MJ/100 g) had the lowest. Protein content was highest in the AIN-93M group, followed by HFDPRS > HFD > HFDCS. However,

the AIN-93M diet was characterized by 70.1% lower fat content compared to the HFD diet.

The HFD, or a high-fat (atherogenic) diet, is a modified AIN-93M diet containing the addition of lard. Lard was added to the diet to induce inflammation in the rat's body. The addition of lard to an atherogenic diet may cause inflammation in rats, as confirmed by Molska et al. [21]. The CRP index in the group with the addition of lard was higher than in the AIN control group [21]. Overall, a high-fat diet triggered the development of metabolic syndrome, which includes oxidative stress, the onset of atherogenic dyslipidemia, pro-inflammatory and pro-thrombotic states, high blood pressure, central obesity, and cardiovascular disease [23].

Energy value, fat, protein, and carbohydrate content were statistically significant different between all diets. The addition of lard increased the amount of fat in the high-fat diets compared to the AIN-93M diet. The HFDPRS diet was characterized by a statistically lower content of carbohydrates compared to the HFDCS diet. It was found that the addition of sprouts increased the carbohydrate content in the groups fed with the HFDCS and HFDPRS diets compared to the group fed with the HFD diet. Noticeable differences in the carbohydrate content in the diets tested could result from yeast activity, which can break down starch [24]. In Molska et al. [21], starch content in modified buckwheat sprouts was lower than in the control sprouts. However, what should be emphasized is that the amount of resistant starch was higher [22].

The type of nutrition has a significant impact on the nutritional status. In the present study, the digestibility of rats was assessed between 19 and 28 days and the data obtained are presented in Table 1 and Fig. 1.

All the rats were weighed at the beginning of the 10-day digestibility study period. The rats that consumed the HFD diet had the lowest body weight, followed by those in the AIN-93M-R, HFDCS-R, and HFDPRS-R groups, respectively. The greatest weight gain was observed in the AIN-93M-R control group, followed by the HFDPRS-R, HFDCS-R and HFD-R groups. During the experiment, rat feces were collected daily, and at the end of the experiment, it was weighed and dried. It was found that the smallest amount of fecal dry matter was excreted by rats consuming the AIN-93M diet. This value was 68.86% lower than the rats consuming the HFDPRS diet. In the case of fecal water content, the highest water content was found in the faeces of rats consuming the diet without any additives, and the lowest in rats consuming the HFDPRS diet (45.40% lower value).

A statistically significant difference in dietary consumption was observed between the AIN-93M-R group and the other groups. However, there were no statistically significant differences between the consumption of individual high-fat diets (HFD, HFDCS, HFDPRS).

The energy from the diet consumed was comparable between the study groups and ranged from 3.65–3.78 MJ/10 days of consumption. An intestinal transit study was carried out during the experiment. The times for the individual groups AIN-93M-R, HFD-R, HFDCS-R and HFDPRS-R are presented in Table 1. Feeding the rats a diet with the addition of lyophilisate control sprouts or probiotic-rich sprouts had no adverse effect on body weight gain or food efficiency. On the other hand, a higher ratio of body weight to length was noted in the rats in the groups supplemented with sprouts (Table 1).

Clinical trials and mechanistic studies on isolated as well as extracted fibers showed a promising regulatory effect on

Table 1 Nutritional parameters of animals fed experimental diets

Parameters	Groups of rats			
	AIN-R	HFD-R	HFDCS-R	HFDPRS-R
Initial body weight on the beginning of digestibility (g)	266.13 ± 31.67^{b}	255.63 ± 20.84^{ab}	289.75 ± 21.51^{a}	292.13 ± 16.15^{b}
Food intake (g/10 days)	207.08 ± 16.78^{b}	170.64 ± 15.00^{a}	184.84 ± 8.10^{a}	184.83 ± 4.40^{a}
Energy intake (MJ/10 days)	3.69 ± 0.29^{a}	3.65 ± 0.32^{a}	3.76 ± 0.16^{a}	3.78 ± 0.09^{a}
FER	0.17 ± 0.05^{a}	0.17 ± 0.03^{a}	0.18 ± 0.03^{a}	0.18 ± 0.03^{a}
Weight-to-length ratio (g/cm)	12.82 ± 0.81^{ab}	12.00 ± 0.87^{a}	13.39 ± 0.73^{b}	13.46 ± 0.40^{b}
Body weight gain (g/10 days)	36.25 ± 13.30^{a}	29.63 ± 7.10^{a}	32.50 ± 6.00^{a}	33.63 ± 5.83^{a}
Fecal excretion (g/10 days)	8.81 ± 0.57^{a}	24.40 ± 4.15^{b}	26.08 ± 3.20^{b}	23.65 ± 1.03^{b}
Fecal excretion (g dry matter/10 days)	6.15 ± 0.44^{a}	20.20 ± 3.12^{b}	20.23 ± 2.65^{b}	19.75 ± 0.76^{b}
Water excretion in feces (g/10 days)	30.00 ± 0.01^{c}	16.66 ± 0.01^{a}	21.99 ± 0.04^{b}	16.38 ± 0.02^{a}
Passage time (min)	791.50 ± 111.00^{a}	687.50 ± 28.00^{a}	774.00 ± 125.00^{a}	752.50 ± 111.00^{a}

Data are mean ± standard deviation. Values with the same superscript letter in each row are not significantly different ($P \leq 0.05$). *AIN-R* A group of rats fed the AIN-93M diet; *FER* Food efficiency ratio; *HFD-R* The group fed the HFD diet; *HFDCS-R* A group of rats fed the HFDCS diet; *HFDPRS-R* A group of rats fed the HFDPRS diet

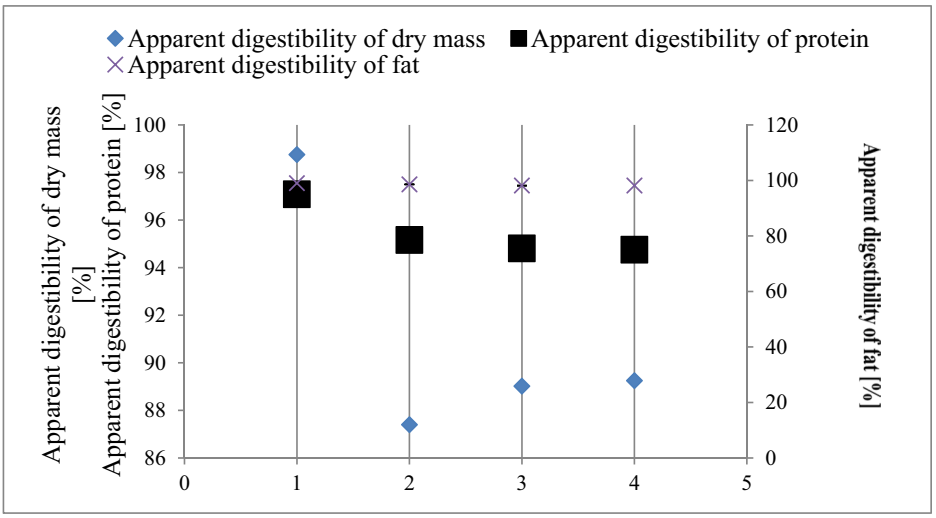

Fig. 1 Determination of digestibility indexes. Data are mean ± standard deviation. Values with the same superscript letter in each row are not significantly different ($P \leq 0.05$). 1: AIN-R; 2: HFD-R; 3: HFDCS-R; 4: HFDPRS-R; AIN-R: a group of rats fed the AIN-93M diet; HFD-R: the group fed the HFD diet; HFDCS-R: a group of rats fed the HFDCS diet; HFDPRS-R: a group of rats fed the HFDPRS diet

Parameters	AIN-R	HFD-R	HFDCS-R	HFDPRS-R
Apparent digestibility of dry mater (g/100 g)	98.75 ± 0.01^{c}	87.40 ± 0.02^{a}	89.02 ± 0.01^{ab}	89.25 ± 0.01^{b}
Apparent digestibility of protein (g/100 g)	95.00 ± 0.01^{c}	78.54 ± 0.04^{b}	75.51 ± 0.01^{a}	75.03 ± 0.01^{a}
Apparent digestibility of fat (g/100 g)	99.00 ± 0.01^{c}	98.57 ± 0.01^{b}	98.14± 0.01^{a}	98.14± 0.01^{a}

the gut (*e.g.*, digestion and absorption, transit time, stool formation) [25]. The publications contain information showing the increase in stool weight significantly reduces its passage time [26]. This may be due to the presence of dietary fiber in the raw material. In this study, such a situation was noted. Compared to the AIN-93M-R group, where the total amount of feces was 8.81 g/10 days, the passage in the HFD-R group was significantly shorter and the total amount of feces was 24.4 g/10 days. In diets with lyophilisate of sprouts, the passage time was shorter than in the AIN-R group. The amount of feces in these diets was 26.08 g/10 days in the HFDCS-R group and 23.65 g/10 days in the HFDPRS-R group. The faster movement of undigested food through the gastrointestinal tract reduces the contact with the intestinal walls, reduces the time of action of digestive enzymes, and may also reduce the risk of colorectal cancer, for example. Such a protective effect is demonstrated by the soluble fraction of dietary fiber, a component found in buckwheat. The dietary fiber content is significantly higher in buckwheat seeds compared to amaranth and quinoa, which have fiber levels comparable to those in regular cereals. According to the literature data on Tartary buckwheat, the total content of dietary fiber (TDF) in seeds is 26%, and soluble (SDF) and insoluble (IDF) 0.54% and 24%, respectively [27–29].

In order to characterize the biological properties of probiotic-rich sprouts, their effect on laboratory animals was analyzed based on selected digestibility indexes. Protein digestibility was 95.00 ± 0.01 (%) in the group fed with the AIN-93M diet. In subsequent groups, the values were, respectively: 78.54 ± 0.04% (HFD-R), 75.51 ± 0.01% (HFDCS-R), 75.03 ± 0.01% (HFDPRS-R). There are statistically significant changes between the food groups of diets with added sprouts and HFD and AIN-93M.

Table 2 Influence of the diets with or without probiotic-rich sprouts on morphological parameters in rats

Parameters	Groups of rats			
	AIN-R	HFD-R	HFDCS-R	HFDPRS-R
WBC G/l	5.70 ± 1.60^{b}	4.18 ± 1.00^{ab}	3.91 ± 0.94^{a}	4.10 ± 1.30^{ab}
NEU G/l	0.44 ± 0.08^{a}	0.34 ± 0.10^{a}	0.33 ± 0.07^{a}	0.32 ± 0.12^{a}
LYM G/l	5.12 ± 1.67^{b}	3.45 ± 0.70^{ab}	3.37 ± 0.87^{a}	3.63 ± 1.19^{ab}
MONO G/l	0.21 ± 0.07^{b}	0.19 ± 0.07^{ab}	0.17 ± 0.07^{ab}	0.11 ± 0.04^{a}
EOS G/l	0.01 ± 0.02^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}
BASO G/l	0.06 ± 0.02^{c}	0.02 ± 0.02^{a}	0.03 ± 0.03^{ab}	0.05 ± 0.02^{bc}
RBC T/l	7.89 ± 0.14^{b}	7.47 ± 0.25^{a}	7.50 ± 0.33^{a}	7.53 ± 0.30^{a}
HGB g/l	146.00 ± 4.41^{b}	139.88 ± 4.22^{ab}	137.50 ± 5.01^{a}	139.25 ± 4.83^{a}
HCT l/l	0.47 ± 0.02^{b}	0.45 ± 0.01^{ab}	0.44 ± 0.02^{a}	0.45 ± 0.02^{ab}
MCV fl	59.28 ± 1.69^{a}	60.76 ± 1.44^{a}	59.23 ± 1.53^{a}	59.6 ± 1.14^{a}
MCH pg	18.51 ± 0.40^{a}	18.74 ± 0.55^{a}	18.35 ± 0.49^{a}	18.51 ± 0.53^{a}
MCHC g/l	312.25 ± 3.54^{a}	308.13 ± 6.24^{a}	309.75 ± 3.54^{a}	310.75 ± 5.90^{a}
PLT G/l	862.38 ± 174.61^{a}	835.17 ± 95.09^{a}	891.00 ± 143.59^{a}	926.86 ± 70.80^{a}

Data are mean ± standard deviation. Values with the same superscript letter in each row are not significantly different ($P \leq 0.05$). *AIN-R* A group of rats fed the AIN-93M diet; *BASO* Basophils; *EOS* Eosinophils; *HCT* Hematocrit; *HFD-R* The group fed the HFD diet; *HFDCS-R* A group of rats fed the HFDCS diet; *HFDPRS-R* A group of rats fed the HFDPRS diet; *HGB* Hemoglobin; *LYM* Lymphocytes; *MCH* Mean blood hemoglobin concentration; *MCHC* Mean blood hemoglobin; *MCV* Mean red blood cell volume; *MONO* Monocytes *NEU* Neutrophils; *PLT* Thrombocytes; *RBC* Red blood cells; *WBC* White blood cells

Protein digestibility in the diets supplemented with sprouts decreased by 19.49 g/100 g (HFDCS) and 19.97 g/100 g (HFDPRS) compared to the AIN-93M diet.

Fat digestibility is lower in the groups with the addition of sprouts compared to the AIN-93M and HFD diets (statistically significant difference).

Table 2 shows the effect of feeding rats with a diet with the addition of lyophilisates of sprouts on blood morphological parameters. There were slight changes in the levels of these parameters.

In the case of rats in the AIN-R group, most morphological parameters were lower in the HFD-R, HFDCS-R, and HFDPRS-R groups. Morphology allows the health status of rats to be assessed. It focuses primarily on the system of red, white blood cells, platelets and the so-called erythrocyte indices. In the HFDPRS-R and HFDCS-R groups, compared to the HFD-R and AIN-93M groups, a decrease was noted in the WBC index, which indicates the number of leukocytes. This may suggest, inter alia, stress and malnutrition.

Changes in the number of red blood cells and hemoglobin in the groups fed the atherogenic diet with the AIN-93M diet may indicate possible anemia in the rats. Taking into account the information presented above, it should be noted that the addition of the lyophilisate of probiotic sprouts could be a factor that improved the values of morphological parameters in the HFDPRS-R group, compared to the rats fed a high-fat diet (HFD-R).

Conclusion

These results show that feeding the rats an atherogenic diet with the addition of probiotic-rich sprouts did not affect weight gain or nutritional efficiency. In the parameters of fat digestibility, there are statistically significant differences between rats fed with the addition of sprouts and those fed with AIN-93M and HFD diets. In addition, diet with lyophilized probiotic-rich sprouts influenced the values of morphological parameters.

Therefore, research should be extended to confirm the impact of modified buckwheat sprouts on digestibility, *e.g.*, in humans. The current trend of searching for new functional raw materials offers many opportunities for both technologists and dieticians. This research gives a new direction for the use of buckwheat sprouts as the raw material.

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Data Availability Not applicable.

Declarations

Ethics Approval Research involving Animals. The animal study protocol was approved by the local Animal Research Ethics Committee in Poznan (Approval no. 28/2017).

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Competing Interests The authors declare no competing interests.

Conflict of Interest The authors declare that they have no conflict of interest.

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Effect of the Addition of Buckwheat Sprouts Modified with the Addition of *Saccharomyces cerevisiae* var. *boulardii* to an Atherogenic Diet on the Metabolism of Sterols, Stanols and Fatty Acids in Rats

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Abstract: The aim of the study was to evaluate the effect of the addition of *Fagopyrum esculentum* Moench buckwheat sprouts modified with the addition of *Saccharomyces cerevisiae* var. *boulardii* to an atherogenic diet on the metabolism of sterols and fatty acids in rats. It was noticed in the study that the group fed with modified sprouts (HFDPRS) had a greater amount of sterols by 75.2%, compared to the group fed on an atherogenic diet (HFD). The content of cholesterol in the liver and feces was lower in the HFDPRS group than the HFD group. In the serum of the HFDPRS group, a more significant amount of the following acids was observed: C18:2 (increase by 13.5%), C20:4 (increase by 15.1%), and C22:6 (increase by 13.1%), compared to the HFDCS group. Regarding the biochemical parameters, it was noted that the group fed the diet with the addition of probiotic-rich sprouts diet had lower non-HDL, LDL-C and CRP ratios compared to the group fed the high-fat diet. The obtained results indicate that adding modified buckwheat sprouts to the diet by adding the probiotic strain of the yeast may have a significant impact on the metabolism of the indicated components in the organism.

Keywords: pseudocereal; probiotic yeast; phytocompounds; cholesterol; lipid profile; in vivo; in vitro

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1. Introduction

Currently, civilization diseases are taking the size of a pandemic. Due to their global spread, they constitute one of the major economic and social health problems. The pathogenesis of most diseases is complex and multifactorial, but a common, important role is played by environmental factors, as well as an inappropriate lifestyle, i.e., an improperly balanced and high-energy diet and limitation of daily physical activity. A diet that consists of highly processed food is low in biologically active ingredients [1]. That is why it is so important to develop novel foods to prevent disease or support therapy and improve the health of patients. Such foods must meet the quality requirements, both chemical and physical, and microbiological requirements, for food products to be safe in its use.

A well-known modifiable risk factor for atherosclerosis is elevated low-density lipoprotein (LDL) cholesterol. It can be lowered by changing the fatty acid composition of the diet. It is also possible to lower LDL cholesterol by consuming products enriched with plant sterols or stanols [2].

Plant sterols (phytosterols) are functional and structural analogs of cholesterol. They are synthesized by plants and are components of plant cell membranes (reducing the fluidity, especially of the surface layer). They are 28 or 29 carbon polycyclic alcohols. Phytosterols have the same polycyclic system as cholesterol, with one hydroxyl group. The difference in construction is in the side chain. Phytosterols are richer with an ethyl or methyl group. They may additionally contain one or two double bonds in this chain. They are divided into the following three main groups: (1) sterols (Δ5-sterols, containing a double bond between C5 and C6; (2) Δ7-sterols with a double bond between C7 and C8, less common in nature); (3) stanols (no double bond in the molecule). In their natural state, these compounds exist in free form. They may also appear as sterol or stanol esters of fatty acids, hydroxycinnamic acid, glucose and glycolipids [3,4].

Buckwheat is a pseudocereal that belongs to the *Polygonaceae* family and includes 15 species growing in the temperate climate of Europe and Asia [5]. The most popular varieties are common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.). Since antiquity, common buckwheat has been cultivated for food and as a medicinal and honey plant [6]. Buckwheat has a healing effect in chronic diseases, such as cancer, diabetes and neurodegenerative diseases, mainly due to bioactive compounds and a well-balanced amino acid pattern [7]. The presence of health-promoting substances in buckwheat makes it more and more often used in various industries, including food, pharmaceutical and cosmetics [8].

The germination process leads to significant changes in the biochemical composition of whole grains. The nitrogen-containing fractions shift towards oligopeptides and free amino acids, and the amino acid composition also changes. In turn, the triacylglycerols begin to hydrolyze and the ratio of saturated to unsaturated fatty acids increases. Starch reserves are mobilized by the action of α-amylase, which corrodes the granular surface and creates holes. The amount of anti-nutritional compounds, i.e., trypsin inhibitor, phytates, and tannins, is significantly reduced. On the other hand, bioactive compounds, such as phenols, phytosterols, folates and GABA, are increased, as a result of which almost all the nutrients in germinating grains are fully available, while various antioxidant compounds are found in higher concentrations. This lays the groundwork for defining sprouts as "functional food" [9].

Consumers are increasingly choosing products that are fresh, highly nutritious, rich in flavor and healthy, including ready-to-eat or ready-to drink foods and beverages. Microbiological trends in food primarily include the use of starter cultures, which can perform many functions (e.g., reduction in toxic compounds, probiotic activity, vitamin production) [10,11]. Probiotics can be used as part of the products that will be their carriers. These are "live strains of carefully selected microorganisms that, when administered in appropriate amounts, provide health benefits to the host". They can affect both the organoleptic and microbiological quality of food. They are used, for example, in traditional food products, such as fermented milk products. Another possibility of using probiotic microorganisms is to modify the raw material by adding them [12,13].

Functional food "has scientifically proven specific health benefits (health claim) beyond their nutritional properties, but the consumption of its specific composition is not essential to human life. Functional food presentation format is food or derived products such as fortified drinks, juices, milk, yoghurts, margarines, cereals, etc." [14]. In the study by Świeca et al. 2018, *Lactobacillus plantarum* 299 V legume sprouts were inoculated. This was carried out in order to produce a new functional product. It has been found that legume sprouts are a good source of nutrients [15]. Another study also used sprouts from legumes (lentils and adzuki beans). They were enriched with the addition of *Saccharomyces cerevisiae* var. *boulardii*. The obtained product was characterized by high pro-health and nutritional properties. Taking into account the high content of antioxidant compounds and all aspects related to the documented, beneficial effects of buckwheat components in model and biological systems, a good solution would be to use buckwheat in the production of a new line of food with designed pro-health properties. Buckwheat is gaining more and

more interest as a potential functional food. It is noted that buckwheat enriched products, as well as the raw material itself, are associated with health benefits [14,16]. Hence, there are hypotheses that (1) adding a probiotic strain may change the composition of the basic raw material; (2) adding modified buckwheat sprouts to an atherogenic diet may improve the lipid profile.

The study was undertaken to evaluate the effect of the addition of buckwheat sprouts modified with the addition of *Saccharomyces cerevisiae* var. *boulardii* to an atherogenic diet on the metabolism of sterols, stanols and fatty acids in rats.

2. Results

2.1. Selected General Nutritional Parameters and Biochemical Parameters

During the experiment, rats were fed the following four diets: basic diet AIN-93M (AIN group), basic diet AIN-93M with the addition of lard (HFD group), basic diet with the addition of lard and lyophilisate of control sprouts (HFDCS group), basic diet with the addition of lard and lyophilisate of modified sprouts (HFDPRS).

Table 1 shows some general nutritional parameters of the rats on experimental diets. The AIN group (970.31 ± 102.20 g) consumed the most significant amount of the diet during the experiment. The remaining groups had amounts comparable to each other, i.e., 669.06 ± 64.68 g (HFD), 674.88 ± 29.25 g (HFDCS) and 671.74 ± 15.03 g (HFDPRS). There is a greater weight gain in the AIN group. However, the lowest gain was in the HFD group. In the HFDCS and HFDPRS groups, the values of weight gain were similar.

Table 1. Selected general nutritional parameters of rats on experimental diets.

Groups	*n*	Parameters			
		Consumed Diet (g)	FER *	Initial Weight (g)	Weight Gain (g)
AIN	8	970.31 ± 102.20 ^{b}	0.20 ± 0.02 ^{a}	188.88 ± 23.71 ^{a}	190.00 ± 26.27 ^{b}
HFD	8	669.06 ± 64.68 ^{a}	0.20 ± 0.03 ^{a}	191.63 ± 12.21 ^{a}	134.88 ± 24.47 ^{a}
HFDCS	8	674.88 ± 29.25 ^{a}	0.25 ± 0.02 ^{b}	187.50 ± 14.36 ^{a}	171.50 ± 9.68 ^{b}
HFDPRS	8	671.74 ± 15.03 ^{a}	0.26 ± 0.02 ^{b}	187.13 ± 15.09 ^{a}	176.75 ± 14.27 ^{b}

* FER (food efficiency ratio) = weight gain (g)/food intake (g) × 100. AIN—group fed with the basic diet AIN-93M; HFD—group fed with the basic diet AIN 93M with the addition of lard; HFDCS—group fed with the basic diet AIN-93M with the addition of lard and lyophilisate from control sprouts; HFDPRS—a group fed with the basic diet AIN-93M with the addition of lard and modified buckwheat sprouts lyophilisate. The mean values with different letters in the row are statistically different ($p < 0.05$). "±" indicates standard deviation.

The FER (food efficiency ratio) value differs between the AIN, HFD, and HFDCS, HFDPRS groups. In the groups with the addition of sprouts, the FER value is statistically higher than in the control groups.

Table 2 shows the effect of feeding rats with a diet with the addition of lyophilisates of sprouts on blood biochemical parameters.

The CRP value differed between the groups and decreased in the HFDCS and HFDPRS groups compared to the HFD diet by 55.9%. Taking the AIN-93M diet as a reference, most parameters were reduced in animals compared to the rats fed high-fat diets.

Non-HDL cholesterol values differed statistically between the groups. A higher value of this index was noticed in rats fed with the HFD diet. However, in the groups of rats fed diets with the addition of sprouts, a decrease in the value of this parameter was noted, i.e., 8.71 ± 3.98 mg/dL (HFDCS) and 7.78 ± 3.00 mg/dL (HFDPRS), respectively, compared to the reference group, i.e., HFD.

Figure 1 provides information related to the weight of the liver and the amount of fat in the liver. The high-fat group experienced a reduction in liver weight compared to the rest of the groups. In the HFDCS (5.14 ± 0.04 g/100 g sample) and HFD (4.99 ± 0.10 g/100 g sample) groups, the liver had the highest fat content, followed by HFDPRS (4.74 ± 0.13 g/100 g sample) and AIN (4.44 ± 0.15 g/100 g sample). Statistical analysis was performed for the liver weight and fat content separately.

Table 2. Influence of the diets with or without probiotic-rich sprouts on biochemical parameters in rats.

Parameters	AIN	HFD	HFDCS	HFDPRS
ALT (U/L)	26.30 ± 7.68 ^{a}	26.24 ± 10.38 ^{a}	24.74 ± 8.09 ^{a}	24.54 ± 4.27 ^{a}
AST (U/L)	104.73 ± 24.27 ^{a}	101.54 ± 56.28 ^{a}	96.56 ± 23.58 ^{a}	117.91 ± 45.75 ^{a}
TCH (mg/dL)	66.86 ± 3.13 ^{a}	79.25 ± 10.86 ^{b}	70.35 ± 6.94 ^{a}	70.19 ± 8.10 ^{ab}
HDL-C (mg/dL)	60.30 ± 2.83 ^{a}	64.74 ± 8.37 ^{a}	61.64 ± 5.83 ^{a}	62.41 ± 7.79 ^{a}
Non-HDL (mg/dL)	7.17 ± 1.46 ^{a}	14.51 ± 5.57 ^{b}	8.71 ± 3.98 ^{a}	7.78 ± 3.00 ^{a}
LDL-C (mg/dL)	7.29 ± 1.86 ^{a}	16.13 ± 4.27 ^{b}	11.14 ± 2.88 ^{a}	10.04 ± 2.26 ^{a}
CRP (mg/L)	0.31 ± 0.03 ^{a}	0.68 ± 0.39 ^{b}	0.30 ± 0.01 ^{a}	0.30 ± 0.01 ^{a}
GLU (mg/dL)	7.41 ± 2.25 ^{ab}	4.68 ± 2.29 ^{a}	6.85 ± 2.10 ^{ab}	8.21 ± 2.28 ^{b}
TAG (mg/dL)	96.49 ± 19.36 ^{b}	53.69 ± 15.51 ^{a}	63.54 ± 17.26 ^{a}	66.54 ± 8.57 ^{a}

The mean values with different letters in the row are statistically different ($p < 0.05$). "±" indicates standard deviation. Each group consisted of (n) eight rats. AIN—group fed with the basic diet AIN-93M; HFD—group fed with the basic diet AIN 93M with the addition of lard; HFDCS—group fed with the basic diet AIN-93M with the addition of lard and lyophilisate from control sprouts; HFDPRS—a group fed with the basic diet AIN-93M with the addition of lard and modified buckwheat sprouts lyophilisate.

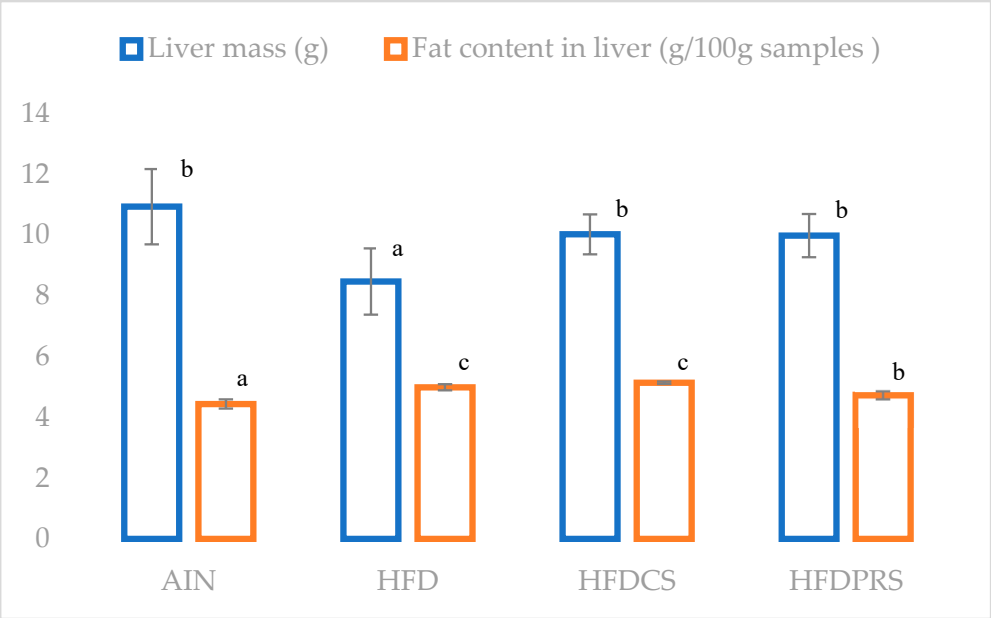

Figure 1. Total fat content and liver mass in experimental groups. Mean values with different letters above the bars differ statistically, $p < 0.05$. Statistical analysis was performed for liver weight and fat content separately. AIN—group fed with the basic diet AIN-93M; HFD—group fed with the basic diet AIN 93M with the addition of lard; HFDCS—group fed with the basic diet AIN-93M with the addition of lard and lyophilisate from control sprouts; HFDPRS—a group fed with the basic diet AIN-93M with the addition of lard and modified buckwheat sprouts lyophilisate.

Figure 2 shows the fat content of seeds, sprouts, diets and feces of the animals. The sprouts in the control group (CS) and probiotic-rich sprouts (PRS) had a higher fat content compared to the seeds. In the case of diets, the HFD diet had the highest fat content, followed by the HFDPRS, HFDCS and AIN diets, respectively. In the case of feces, the HFDPRS-F group had the highest fat content, followed by HFCS-F, HFD-F, and AIN-F, respectively.

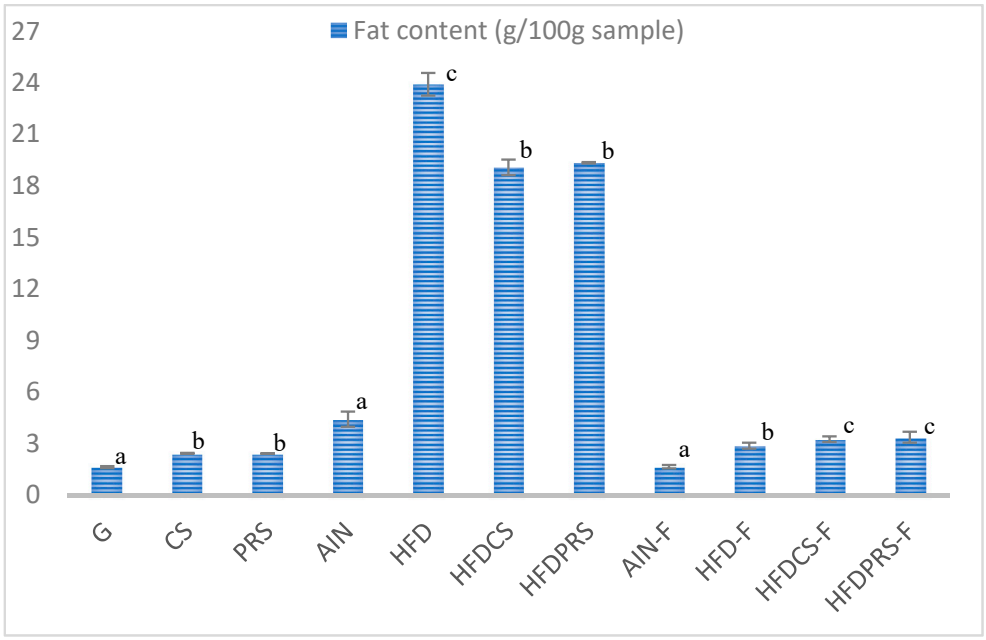

Figure 2. Total fat content in different forms of buckwheat. Mean values with different letters above the bars differ statistically, $p < 0.05$. G—grains, CS—control sprouts, PRS—probiotic-rich sprouts; AIN—group fed with the basic diet AIN-93M; HFD—group fed with the basic diet AIN 93M with the addition of lard; HFDCS—group fed with the basic diet AIN-93M with the addition of lard and lyophilisate from control sprouts; HFDPRS—a group fed with the basic diet AIN-93M with the addition of lard and modified buckwheat sprouts lyophilisate. AIN-F—feces of the AIN group, HFD-F—feces of the HFD group, HFDCS-F—feces of the HFDCS group, HFDPRS-F—feces of the AIN group.

2.2. Sterols

The content of sterols is presented in Table 3. The indicated compounds were identified in seeds, sprouts, diets, livers, feces and serum. A detailed description of the results is presented in the following Sections 2.2.1–2.2.5.

2.2.1. *F. esculentum* Freeze-Dried Grains and Sprouts

Twelve compounds were identified in seeds and sprouts (brassicasterol, campesterol, campestanol, stigmasterol, β-sitosterol, sitostanol, Δ5-avenasterol, α-amyrin, 5,24-stigmastadienol, Δ7-stigmasterol, cycloartanol, 24-methylenecycloartanol). Seeds and control sprouts as well as probiotic-rich sprouts were characterized by the highest content of β-sitosterol, followed by Δ5-avenasterol and campesterol. The highest sterol content characterized the seeds, followed by control sprouts < probiotic-rich sprouts.

2.2.2. Experimental Diets

Eleven compounds were provided with the diet. The highest amount of sterols was provided by the diet AIN (2102.4 ± 12.74 μg/g fat), then HFDPRS (394.64 ± 0.59 μg/g fat), HFDCS (246.42 ± 5.57 μg/g fat), HFD (97.86 ± 0.97 μg/g fat).

2.2.3. Serum

Cholesterol was identified in the blood serum. The highest amount in 100 μL of serum was found in the AIN-S group, and then in the HFD-S, HFDPRS-S and HFDCS-S groups, respectively. It was noticed that the group fed the diet with the addition of modified buckwheat sprouts had 48.1% lower cholesterol content, compared to the rats fed the AIN diet.

Sitostanol was also identified in the AIN-S, HFDCS-S and HFDPRS-S groups, with the highest amount in the AIN-S group.

Table 3. The content of sterols in buckwheat seeds (mg/g lipids) and sprouts (mg/g lipids), experimental diets (μg/g lipids), serum (μg/100 μL serum), liver (mg/g lipids) and feces (mg/g lipids).

Sample	Cholesterol	Brassicasterol	Campesterol	Campestanol	Stigmasterol	β-Sitosterol	Sitostanol	Δ5-Avenasterol	α-Amryin	5,24-Stigmasta-Dienol	Δ7-Stigmasterol	Cycloartanol	24-Methylene-cycloartanol	Total Sterols
G	ND	0.30 ± 0.09 ^{a}	1.85 ± 0.42 ^{a}	0.14 ± 0.03 ^{a}	0.35 ± 0.05 ^{a}	13.7 ± 1.80 ^{a}	0.40 ± 0.06 ^{a}	2.51 ± 0.19 ^{b}	0.42 ± 0.09 $_{a}$	0.14 ± 0.02 ^{a}	0.34 ± 0.09 ^{a}	0.20 ± 0.03 ^{a}	0.34 ± 0.03 ^{a}	20.81 ± 1.93 ^{a}
CS	ND	0.30 ± 0.08 ^{a}	1.97 ± 0.47 ^{a}	0.17 ± 0.04 ^{a}	1.52 ± 0.28 ^{b}	12.8 ± 1.24 ^{a}	0.40 ± 0.09 ^{a}	2.31 ± 0.30 $_{ab}$	0.35 ± 0.17 $_{a}$	0.14 ± 0.06 ^{a}	0.41 ± 0.08 ^{a}	0.16 ± 0.03 ^{a}	0.26 ± 0.05 ^{a}	20.85 ± 1.88 ^{a}
PRS	ND	0.31 ± 0.08 ^{a}	1.69 ± 0.36 ^{a}	0.16 ± 0.04 ^{a}	1.48 ± 0.26 ^{b}	11.67 ± 1.47 $_{a}$	0.38 ± 0.07 ^{a}	2.00 ± 0.36 ^{a}	0.31 ± 0.11 $_{a}$	0.18 ± 0.05 ^{a}	0.32 ± 0.07 ^{a}	0.20 ± 0.07 ^{a}	0.30 ± 0.09 ^{a}	19.00 ± 2.18 ^{a}
AIN	ND	76.96 ± 4.52 $_{a}$	459.43 ± 0.81 $_{d}$	16.21 ± 0.11 $_{a}$	101.03 ± 0.42 ^{d}	1093.56 ± 9.11 ^{d}	70.09 ± 0.12 $_{d}$	44.89 ± 3.30 $_{c}$	ND	93.11 ± 1.97 $_{c}$	57.06 ± 1.51 $_{c}$	25.20 ± 0.11 $_{c}$	64.92 ± 4.79 $_{c}$	2102.40 ± 12.74 ^{d}
HFD	ND	ND	38.46 ± 1.82 ^{a}	ND	4.37 ± 0.23 ^{a}	35.10 ± 1.23 $_{a}$	2.19 ± 0.05 ^{a,}	2.28 ± 0.18 ^{a}	ND	3.30 ± 0.21 ^{a}	2.78 ± 0.07 ^{a}	5.11 ± 0.42 ^{a}	4.29 ± 0.35 ^{a}	97.86 ± 0.97 ^{a}
HFDCS	ND	ND	65.94 ± 3.26 ^{b}	ND	10.48 ± 0.74 $_{b}$	108.99 ± 1.44 ^{b}	4.12 ± 0.17 ^{b}	14.71 ± 0.48 $_{b}$	ND	7.43 ± 0.05 ^{b}	8.85 ± 0.14 ^{b}	18.92 ± 0.51 $_{b}$	6.99 ± 0.49 ^{a}	246.42 ± 5.57 $_{b}$
HFDPRS	ND	ND	96.68 ± 5.63 ^{c}	ND	16.06 ± 0.11 $_{c}$	179.40 ± 3.01 ^{c}	9.43 ± 0.32 ^{c}	20.71 ± 0.38 $_{b}$	ND	8.76 ± 0.04 ^{b}	10.75 ± 0.04 $_{b}$	30.96 ± 0.64 $_{d}$	21.90 ± 1.56 $_{b}$	394.64 ± 0.59 $_{c}$
AIN-S	49.00 ± 1.31 $_{b}$	ND	ND	ND	ND	ND	1.23 ± 0.18 ^{b}	ND	ND	ND	ND	ND	ND	47.78 ± 3.06 ^{b}
HFD-S	26.58 ± 1.04 $_{a}$	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	26.58 ± 3.58 ^{a}
HFDCS-S	19.38 ± 1.10 $_{a}$	ND	ND	ND	ND	ND	0.64 ± 0.05 ^{a}	ND	ND	ND	ND	ND	ND	20.03 ± 5.43 ^{a}
HFDPRS-S	25.45 ± 0.52 $_{a}$	ND	ND	ND	ND	ND	0.67 ± 0.03 ^{a}	ND	ND	ND	ND	ND	ND	26.12 ± 3.71 ^{a}
AIN-L	28.4 ± 2.04 $_{ab}$	ND	0.35 ± 0.08 ^{b}	0.11 ± 0.00 ^{c}	0.07 ± 0.00 $_{bc}$	0.57 ± 0.05 ^{c}	0.01 ± 0.00 ^{a,}	0.08 ± 0.00 $_{bc}$	ND	ND	ND	ND	ND	29.64 ± 2.06 $_{ab}$
HFD-L	32.32 ± 2,76 $_{b}$	ND	0.14 ± 0.04 ^{a}	0.04 ± 0.00 ^{a}	0.09 ± 0.02 ^{c}	0.17 ± 0.02 ^{a}	ND	0.09 ± 0.01 ^{c}	ND	ND	ND	ND	ND	32.84 ± 2.75 ^{b}
HFDCS-L	31.20 ± 3.14 $_{b}$	ND	0.20 ± 0.07 ^{a}	0.07 ± 0.01 ^{b}	0.04 ± 0.01 ^{a}	0.40 ± 0.07 ^{b}	0.01 ± 0.00 ^{a}	0.05 ± 0.01 ^{a}	ND	ND	ND	ND	ND	31.93 ± 3.11 ^{b}
HFDPRS-L	24.68 ± 1.96 $_{a}$	ND	0.13 ± 0.05 ^{a,}	0.07 ± 0.01 ^{b}	0.05 ± 0.01 $_{ab}$	0.38 ± 0.03 ^{b}	ND	0.07 ± 0.01 ^{b}	ND	ND	ND	ND	ND	25.30 ± 1.98 ^{a}
AIN-F	15.45 ± 2.25 $_{a}$	12.81 ± 1.81 $_{b}$	0.64 ± 0.01 ^{a}	ND	ND	5.27 ± 0.37 $_{ab}$	1.56 ± 0.11 ^{b}	0.11 ± 0.00 ^{a}	ND	ND	0.97 ± 0.04 $_{b,}$	0.78 ± 0.08 $_{ab}$	1.38 ± 0.15 ^{b}	38.96 ± 4.64 ^{a}
HFD-F	52.04 ± 1.73 $_{b}$	5.40 ± 0.88 ^{a}	2.37 ± 0.11 ^{b}	0.83 ± 0.08 ^{a}	1.33 ± 0.23 ^{c}	5.55 ± 0.07 ^{b}	1.12 ± 0.03 ^{a}	0.33 ± 0.03 ^{b}	ND	1.52 ± 0.02 ^{c}	0.62 ± 0.03 ^{b}	0.48 ± 0.06 ^{a}	1.14 ± 0.22 $_{ab}$	72.72 ± 3.29 ^{b}
HFDCS-F	13.53 ± 0.85 $_{a}$	26.98 ± 1.00 $_{d}$	5.16 ± 0.19 ^{d}	1.00 ± 0.00 ^{b}	0.45 ± 0.01 $_{ab}$	5.77 ± 0.08 ^{b}	2.10 ± 0.14 ^{c}	1.97 ± 0.04 ^{d}	0.55 ± 0.14 $_{a}$	1.15 ± 0.04 ^{b}	1.15 ± 0.01 ^{c}	1.02 ± 0.15 ^{b}	1.45 ± 0.11 ^{b}	62.27 ± 1.92 ^{b}
HFDPRS-F	9.47± 0.00 ^{a}	20.13 ± 0.17 $_{c}$	3.15 ± 0.23 ^{c}	ND	0.60 ± 0.04 ^{b}	4.53 ± 0.07 ^{a}	1.39 ± 0.10 $_{ab}$	1.35 ± 0.00 ^{c}	0.44 ± 0.04 $_{a}$	0.69 ± 0.02 ^{a}	0.65 ± 0.03 ^{a}	0.73 ± 0.07 $_{ab}$	0.78 ± 0.01 ^{a}	43.90 ± 0.26 ^{a}

ND—not detected. The mean values with different letters in the column in each group are statistically different ($p < 0.05$). "±" indicates standard deviation. G—grains, CS—control sprouts, PRS—probiotic-rich sprouts; AIN-L—liver of the AIN group, HFD-L—liver of the HFD group, HFDCS-L—liver of the HFDCS group, HFDPRS-L—liver of the HFDPRS group, AIN-F—feces of the AIN group, HFD-F—feces of the HFD group, HFDCS-F—feces of the HFDCS group, HFDPRS-F—feces of the AIN group, AIN—group fed with the basic diet AIN-93M; HFD—group fed with the basic diet AIN 93M with the addition of lard; HFDCS—group fed with the basic diet AIN-93M with the addition of lard and lyophilisate from control sprouts; HFDPRS—a group fed with the basic diet AIN-93M with the addition of lard and modified buckwheat sprouts lyophilisate, AIN-S—serum of rats of the AIN group, HFD-S—serum of rats of the HFD group, HFDCS-S—serum of rats of the HFDCS group, HFDPRS-S—serum of rats of the HFDPRS group.

2.2.4. Liver

Seven compounds were identified in the livers. Moreover, in the total content of liver sterols, cholesterol had the highest content.

Cholesterol content decreased, respectively, in the following groups: HFD-L (32.32 ± 2.76 mg/g fat), HFDCS-L (31.20 ± 3.14 mg/g fat), AIN-L (28.44 ± 2.04 mg/g fat), HFDPRS-L (24.68 ± 1.96 mg/g fat). It should be noted that the group of rats fed with the diet containing freeze-dried modified sprouts had a significantly lower content of this compound compared to the groups of HFD-L (by 23.6%) and HFDCS-L (by 20.9%).

2.2.5. Feces

Thirteen compounds were identified in the feces, of which campestanol, stigmasterol, α-amyrin, 5,24-stigmastadienol were identified only in selected groups.

Among the identified sterols, cholesterol had the largest share in the total content of sterols in the AIN-F and HFD-F groups. The highest amount was determined in the feces of the HFD-F group (52.04 ± 1.73 mg/g fat), then AIN-F (15.45 ± 2.25 mg/g fat), HFDCS-F (13.53 ± 0.85 mg/g fat), HFDPRS-F (9.47 ± 0.00 mg/g fat). It was found that the group fed the diet with modified buckwheat sprouts had 81.8% lower fecal cholesterol content compared to the rats fed the high-fat diet.

There was a high amount of the following compounds in the stool: brassicasterol, β-sitosterol. The highest content of brassicasterol was determined in feces in the HFDCS-F group, and the lowest in the HFD-F group. In the feces of the HFDPRS-F group (26.98 ± 1.00 mg/g fat), the amount of this compound was lower than in the HFDCS-F group (20.13 ± 0.17 mg/g fat).

The amount of β-sitosterol was identified to be the highest in HFDCS-F (5.77 ± 0.08 mg/g fat) and the lowest in the HFDPRS-F group (4.53 ± 0.07 mg/g fat). There were also statistically significant differences between the groups in the amount of campesterol. The HFDCS-F group had the highest content, followed by HFDPRS-F, HFD-F, AIN-F. The HFDCS-F group had the highest content, followed by HFDPRS-F, HFD-F, AIN-F.

The amount of sitostanol in the stool was the lowest in the HFD-F group, then it increased in the following order: HFDPRS-F (by 19.4%), AIN-F (by 28.2%), and HFDCS-F (by 46.7%).

The HFDPRS-F group was characterized by the lowest content in feces of the following compounds: 5,24-stigmastadienol, 24-methylenecycloartanol, Δ7-stigmasterol, 5,24-stigmastadienol, stigmasterol, cholesterol.

Among the study groups, the HFD-F group had the highest total fecal sterol content. The value was higher than in the HFDPRS-F group by 39.6%.

2.3. Fatty Acids

Table 4 shows the identified fatty acids in seeds, sprouts, diets, livers, feces and serum. In contrast, Table 5 lists the amounts of saturated fatty acids (SFA), monoenoic (MUFA) and polyene fatty acids (PUFA). A detailed description of the results is presented in Sections 2.2.2, 2.3.1, 2.3.3 and 2.3.4.

Table 4. The content of fatty acids in buckwheat seeds (mg/g lipids) and sprouts (mg/g lipids), experimental diets (mg/g lipids), serum (μg/100 μL serum), liver (mg/g lipids) and feces (mg/g lipids).

Fatty Acids	G	CS	PRS	AIN	HFD	HFDCS	HFDPRS	AIN-S	HFD-S	HFDCS-S	HFDPRS-S	AIN-L	HFD-L	HFDCS-L	HFDPRS-L	AIN-F	HFD-F	HFDCS-F	HFDPRS-F
C10:0	ND	ND	ND	ND	ND	ND	ND	46.38 ± 3.12 ^{b}	34.99 ± 2.34 ^{a}	32.62 ± 2.21 ^{a}	32.11 ± 2.49 ^{a}	ND	ND	ND	ND	ND	ND	ND	ND
C14:0	ND	0.72 ± 0.02 ^{a}	1.53 ± 0.06 ^{c}	5.49 ± 0.15 ^{a}	9.77 ± 0.39 ^{b}	9.90 ± 0.24 ^{b}	9.56 ± 0.30 ^{b}	454.36 ± 3.09 ^{c}	366.97 ± 5.75 ^{a}	385.64 ± 5.81 ^{b}	389.95 ± 4.97 ^{b}	2.13 ± 0.16 ^{ab}	1.82 ± 0.05 ^{a}	2.51 ± 0.21 ^{b}	2.30 ± 0.17 ^{ab}	18.48 ± 0.00 ^{c}	2.41 ± 0.23 ^{a}	4.47 ± 0.54 ^{b}	3.88 ± 0.69 ^{b}
C 16:0	127.82 ± 0.20 ^{c}	59.83 ± 0.11 ^{a}	89.91 ± 0.10 ^{b}	89.79 ± 0.93 ^{a}	195.29 ± 0.35 ^{b}	204.26 ± 0.25 ^{d}	199.41 ± 0.32 ^{c}	121.31 ± 4.06 ^{c}	88.18 ± 4.50 ^{a}	86.36 ± 4.77 ^{a}	96.37 ± 2.93 ^{b}	149.26 ± 4.63 ^{a}	140.19 ± 6.48 ^{a}	166.31 ± 3.27 ^{b}	162.09 ± 2.68 ^{b}	132.61 ± 0.00 ^{c}	48.26 ± 0.86 ^{a}	69.25 ± 4.13 ^{b}	62.95 ± 7.22 ^{b}
C18:0	22.52 ± 0.19 ^{c}	10.83 ± 0.19 ^{a}	16.63 ± 0.46 ^{b}	44.69 ± 0.33 ^{a}	111.89 ± 0.32 ^{b}	118.23 ± 0.79 ^{c}	111.89 ± 0.39 ^{b}	84.95 ± 3.07 ^{a}	92.13 ± 2.37 ^{b}	85.62 ± 3.78 ^{a}	93.16 ± 4.31 ^{b}	133.68 ± 3.62 ^{bc}	94.07 ± 4.37 ^{a}	131.25 ± 3.49 ^{b}	141.15 ± 0.86 ^{c}	176.63 ± 0.00 ^{c}	81.72 ± 1.57 ^{a}	120.24 ± 9.68 ^{b}	123.28 ± 4.72 ^{b}
C20:0	11.66 ± 0.38 ^{b}	7.92 ± 0.08 ^{a}	12.36 ± 0.14 ^{b}	2.95 ± 0.15 ^{c}	1.59 ± 0.07 ^{a}	2.14 ± 0.05 ^{b}	2.01 ± 0.06 ^{b}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.82 ± 0.00 ^{a}	4.05 ± 0.00 ^{b}
C22:0	13.86 ± 0.27 ^{b}	9.74 ± 0.23 ^{a}	15.08 ± 0.12 ^{c}	8.50 ± 0.52 ^{b}	0.76 ± 0.05 ^{a}	1.46 ± 0.11 ^{a}	1.49 ± 0.05 ^{a}	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.91 ± 0.00 ^{a}	3.97 ± 0.00 ^{b}
C23:0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	130.27 ± 2.77 ^{a}	142.33 ± 2.33 ^{b}	151.05 ± 1.80 ^{b}	169.71 ± 4.79 ^{c}	ND	ND	ND	ND
C24:0	7.47 ± 0.34 ^{a}	7.16 ± 0.09 ^{a}	10.38 ± 0.00 ^{b}	2.96 ± 0.06 ^{c}	0.31 ± 0.02 ^{a}	0.64 ± 0.00 ^{b}	0.59 ± 0.01 ^{b}	1.99 ± 0.19 ^{b}	1.47 ± 0.16 ^{a}	1.79 ± 0.19 ^{b}	1.51 ± 0.10 ^{a}	ND	ND	ND	ND	ND	ND	ND	ND
C14:1	ND	ND	ND	ND	ND	ND	ND	22.58 ± 2.05 ^{b}	20.65 ± 0.46 ^{b}	17.98 ± 2.06 ^{a}	16.56 ± 1.90 ^{a}	ND	ND	ND	ND	ND	ND	ND	ND
C16:1	ND	1.59 ± 0.09 ^{a}	2.24 ± 0.05 ^{b}	1.75 ± 0.05 ^{a}	13.63 ± 0.32 ^{b}	13.64 ± 0.51 ^{b}	13.29 ± 0.77 ^{b}	11.83 ± 0.53 ^{b}	3.71 ± 0.28 ^{a}	4.08 ± 0.37 ^{a}	4.04 ± 0.25 ^{a}	4.23 ± 0.64 ^{a}	12.75 ± 0.02 ^{b}	5.19 ± 0.40 ^{a}	4.87 ± 0.49 ^{a}	ND	1.82 ± 0.25 ^{b}	1.07 ± 0.24 ^{ab}	0.66 ± 0.53 ^{a}
C18:1	313.47 ± 0.32 ^{c}	169.33 ± 2.13 ^{a}	262.34 ± 0.44 ^{b}	369.55 ± 0.35 ^{d}	322.09 ± 0.23 ^{a}	349.13 ± 0.82 ^{c}	339.84 ± 0.44 ^{b}	91.88 ± 3.73 ^{c}	78.77 ± 2.44 ^{ab}	75.32 ± 4.11 ^{a}	83.52 ± 3.36 ^{b}	174.32 ± 8.08 ^{b}	112.57 ± 5.33 ^{a}	180.56 ± 0.38 ^{b}	170.17 ± 1.05 ^{b}	40.76 ± 0.00 ^{a}	91.88 ± 1.86 ^{d}	74.96 ± 4.68 ^{c}	56.72 ± 4.63 ^{b}
C18:1 trans	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	30.98 ± 0.00 ^{c}	10.89 ± 1.00 ^{ab}	10.99 ± 1.51 ^{b}	8.21 ± 0.97 ^{a}
C20:1	23.3 ± 0.07 ^{b}	13.89 ± 0.69 ^{a}	22.24 ± 0.28 ^{b}	1.97 ± 0.09 ^{a}	4.91 ± 0.14 ^{b}	6.24 ± 0.03 ^{d}	5.77 ± 0.00 ^{c}	3.58 ± 0.07 ^{b}	3.26 ± 0.24 ^{a}	3.46 ± 0.27 ^{ab}	3.95 ± 0.14 ^{c}	2.51 ± 0.31 ^{b}	ND	2.19 ± 0.08 ^{b}	2.21 ± 0.09 ^{b}	18.48 ± 0.00 ^{c}	1.68 ± 0.23 ^{a}	3.81 ± 0.23 ^{b}	3.53 ± 0.38 ^{b}
C 18:2	316.06 ± 0.89 ^{c}	174.21 ± 1.89 ^{a}	270.89 ± 0.67 ^{b}	621.37 ± 1.90 ^{c}	132.19 ± 0.38 ^{a}	149.76 ± 0.05 ^{b}	148.24 ± 0.19 ^{b}	107.35 ± 4.07 ^{c}	61.21 ± 1.73 ^{a}	59.51 ± 3.73 ^{a}	68.78 ± 2.53 ^{b}	110.52 ± 7.09 ^{a}	109.07 ± 3.73 ^{ab}	126.39 ± 6.46 ^{b}	122.07 ± 2.50 ^{ab}	16.85 ± 0.00 ^{d}	7.91 ± 0.53 ^{b}	9.86 ± 0.79 ^{c}	6.48 ± 0.29 ^{a}
C18:3n-3	19.92 ± 0.10 ^{c}	9.67 ± 0.43 ^{a}	14.09 ± 0.08 ^{b}	3.54 ± 0.01 ^{a}	5.51 ± 0.23 ^{b}	5.78 ± 0.19 ^{b}	5.24 ± 0.12 ^{b}	1.91 ± 0.21 ^{b}	1.14 ± 0.15 ^{a}	1.31 ± 0.22 ^{a}	1.75 ± 0.10 ^{b}	1.65 ± 0.32 ^{b}	ND	1.39 ± 0.25 ^{b}	1.21 ± 0.15 ^{b}	ND	ND	ND	ND
C18:3n-6	ND	ND	ND	ND	ND	ND	ND	2.17 ± 0.17 ^{c}	1.63 ± 0.15 ^{b}	1.20 ± 0.14 ^{a}	1.55 ± 0.12 ^{b}	2.59 ± 0.12 ^{a}	2.78 ± 0.09 ^{ab}	2.85 ± 0.21 ^{ab}	3.13 ± 0.20 ^{b}	ND	ND	ND	ND
C20:3n-6	ND	ND	ND	ND	ND	ND	ND	1.55 ± 0.11 ^{b}	1.05 ± 0.10 ^{a}	1.50 ± 0.22 ^{b}	1.49 ± 0.10 ^{b}	ND	ND	ND	ND	ND	ND	ND	ND
C20:4n-6	ND	ND	ND	ND	ND	ND	ND	136.18 ± 5.08 ^{b}	137.77 ± 3.54 ^{b}	118.35 ± 4.35 ^{a}	139.44 ± 4.23 ^{b}	ND	ND	ND	ND	ND	ND	ND	ND

Table 4. *Cont.*

Fatty Acids	G	CS	PRS	AIN	HFD	HFDCS	HFDPRS	AIN-S	HFD-S	HFDCS-S	HFDPRS-S	AIN-L	HFD-L	HFDCS-L	HFDPRS-L	AIN-F	HFD-F	HFDCS-F	HFDPRS-F
C22:5	ND	ND	ND	ND	ND	ND	ND	1.17 ± 0.06 ^{a}	1.76 ± 0.15 ^{c}	1.50 ± 0.15 ^{b}	1.86 ± 0.11 ^{c}	ND	ND	ND	ND	ND	ND	ND	ND
C22:6	ND	ND	ND	ND	ND	ND	ND	4.45 ± 0.35 ^{a}	10.55 ± 0.34 ^{d}	7.69 ± 0.46 ^{b}	8.85 ± 0.50 ^{c}	ND	ND	ND	ND	ND	ND	ND	ND

ND—not detected. The mean values with different letters in the row in each group are statistically different ($p < 0.05$). "±" indicates standard deviation. G—grains, CS—control sprouts, PRS—probiotic-rich sprouts; AIN-L—liver of the AIN group, HFD-L—liver of the HFD group, HFDCS-L—liver of the HFDCS group, HFDPRS-L—liver of the HFDPRS group, AIN-F—feces of the AIN group, HFD-F—feces of the HFD group, HFDCS-F—feces of the HFDCS group, HFDPRS-F—feces of the AIN group, AIN—group fed with the basic diet AIN-93M; HFD—group fed with the basic diet AIN 93M with the addition of lard; HFDCS—group fed with the basic diet AIN-93M with the addition of lard and lyophilisate from control sprouts; HFDPRS—a group fed with the basic diet AIN-93M with the addition of lard and modified buckwheat sprouts lyophilisate, AIN-S—serum of rats of the AIN group, HFD-S—serum of rats of the HFD group, HFDCS-S—serum of rats of the HFDCS group, HFDPRS-S—serum of rats of the HFDPRS group.

Table 5. The amount of individual groups of fatty acids (SFA, MUFA, PUFA) in buckwheat seeds (mg/g lipids) and sprouts (mg/g lipids), experimental diets (mg/g lipids), serum (μg/100 μL serum), liver (mg/g lipids) and feces (mg/g lipids).

	G	CS	PRS	AIN	HFD	HFDCS	HFDPRS	AIN-S	HFD-S	HFDCS-S	HFDPRS-S	AIN-L	HFD-L	HFDCS-L	HFDPRS-L	AIN-F	HFD-F	HFDCS-F	HFDPRS-F
SFA	183.33 ± 0.06 ^{c}	96.20 ± 0.12 ^{a}	145.89 ± 0.87 ^{b}	154.38 ± 0.38 ^{a}	319.61 ± 1.19 ^{b}	336.63 ± 2.76 ^{c}	324.95 ± 1.13 ^{b}	708.99 ± 5.81 ^{c}	583.74 ± 9.35 ^{a}	592.03 ± 10.64 ^{a}	613.10 ± 6.82 ^{b}	415.34 ± 9.96 ^{b}	378.41 ± 13.23 ^{a}	451.12 ± 7.95 ^{c}	475.25 ± 5.95 ^{c}	327.72 ± 0.00 ^{c}	132.39 ± 1.91 ^{a}	199.69 ± 6.67 ^{b}	198.13 ± 4.96 ^{b}
MUFA	336.77 ± 0.25 ^{c}	184.81 ± 2.91 ^{a}	286.82 ± 0.21 ^{b}	373.27 ± 0.48 ^{d}	340.63 ± 0.70 ^{a}	369.01 ± 1.35 ^{c}	358.90 ± 1.21 ^{b}	129.87 ± 3.27 ^{c}	106.39 ± 2.24 ^{b}	100.84 ± 4.86 ^{a}	108.07 ± 3.54 ^{b}	181.06 ± 8.91 ^{b}	125.32 ± 5.35 ^{a}	187.94 ± 0.11 ^{b}	177.25 ± 1.43 ^{b}	90.22 ± 0.00 ^{b}	106.27 ± 3.16 ^{c}	90.83 ± 5.99 ^{b}	69.12 ± 5.89 ^{a}
PUFA	335.98 ± 0.99 ^{c}	183.88 ± 1.46 ^{a}	284.98 ± 0.75 ^{b}	624.91 ± 1.90 ^{c}	137.70 ± 0.61 ^{a}	155.54 ± 0.24 ^{b}	153.48 ± 0.07 ^{b}	254.78 ± 4.78 ^{c}	215.11 ± 3.70 ^{b}	191.06 ± 4.67 ^{a}	223.72 ± 5.58 ^{b}	114.76 ± 7.10 ^{a}	111.85 ± 3.82 ^{a}	130.63 ± 6.85 ^{b}	126.41 ± 2.55 ^{ab}	16.85 ± 0.00 ^{d}	7.91 ± 0.53 ^{b}	9.86 ± 0.79 ^{c}	6.48 ± 0.29 ^{a}

The mean values with different letters in the row in each group are statistically different ($p < 0.05$). "±" indicates standard deviation. G—grains, CS—control sprouts, PRS—probiotic-rich sprouts; AIN-L—liver of the AIN group, HFD-L—liver of the HFD group, HFDCS-L—liver of the HFDCS group, HFDPRS-L—liver of the HFDPRS group, AIN-F—feces of the AIN group, HFD-F—feces of the HFD group, HFDCS-F—feces of the HFDCS group, HFDPRS-F—feces of the AIN group, AIN—group fed with the basic diet AIN-93M; HFD—group fed with the basic diet AIN 93M with the addition of lard; HFDCS—group fed with the basic diet AIN-93M with the addition of lard and lyophilisate from control sprouts; HFDPRS—a group fed with the basic diet AIN-93M with the addition of lard and modified buckwheat sprouts lyophilisate, AIN-S—serum of rats of the AIN group, HFD-S—serum of rats of the HFD group, HFDCS-S—serum of rats of the HFDCS group, HFDPRS-S—serum of rats of the HFDPRS group.

2.3.1. *F. esculentum* Freeze-Dried Grains, Sprouts and Experimental Diets

Buckwheat seeds contain a higher content of SFA, MUFA and PUFA, compared to sprouts (Table 5). When comparing the lyophilisate of control sprouts with modified sprouts, it was noticed that the PRS group has a higher content of the indicated groups of fatty acids. Nine fatty acids were identified in seeds, and eleven in control and modified sprouts (Table 4).

As with sprouts, eleven fatty acids have been identified in all diets. Comparing the diets, it was noticed that the HFDCS diet contained the most saturated fatty acids. In the case of monoenoic and polyenic acids, the AIN diet was the highest, followed by the HFDCS, HFDPRS and HFD diets.

Lard, which was part of the diets in the HFD, HFDCS and HFDPRS groups, increased the amount of saturated fatty acids. The lard addition was 200 g/kg of the HFD diet and 200g/1.3 kg of the HFDCS and HFDPRS diets. The differences between the AIN diet and diets with the addition of sprouts were especially noticed in the case of palmitic (C16:0) and stearic (C18:0) acids.

2.3.2. Serum

Among the studied groups, the highest content of saturated fatty acids, monoenic and polyenic fatty acids was found in the AIN-S group (Table 5).

Statistical differences were noticeable when comparing the groups in which sprouts were added to the diets. There is an increase in the total content of monoenoic and polyenic acids in the HFDPRS-S group compared to HFDCS-S (Table 5). The increase was 14.6% and 6.7%, respectively. The following fatty acids C18:1, C20:1, C18:2, C18:3n-3, C18:3n-6, C20:4n-6, C22:5, C22:6 have a significant influence on the differences (Table 4).

2.3.3. Liver

Ten fatty acids were identified in the livers of rats, of which the following acids were not identified in the rats in the HFD-L group: eicosenoic acid (C20:1) and α -linolenic acid (C18:3n-3) (Table 4).

The livers of rats from all groups had a high content of saturated fatty acids. In the case of PUFA, there is a slight increase in the amount in the HFDPRS-L and HFDCS-L groups compared to the AIN-L and HFD L groups. It was influenced by linoleic acid (C18:2) and γ-linolenic acid. (18:3n-6).

2.3.4. Feces

Ten fatty acids (HFDCS-F groups, HFDPRS-F groups) were identified in the rats' feces in the experiment. The following acids were not detected in the HFD-F group: arachidic (C20:0); docosanoic (C22:0). However, in the AIN-F group, no acids were detected, i.e., arachidic (C20:0), docosanoic (C 22:0); palmitolenic acid (C16:1).

The amount of excreted polyenic acids was higher in the AIN-F group by 61.5%, compared to the HFDPRS-F group. In total, the amount of saturated fatty acids in the stool was lower in the HFD-F, HFDCS-F, and HFDPRS-F groups, compared to the liver's content.

3. Discussion

3.1. Sterols

Plant sterols and stanols are effective in lowering serum LDL cholesterol. Hence, they may play an important role in the development of atherosclerotic lesions. Both sterols and plant stanols are useful for hypercholesterolemic patients. They can be in addition to cholesterol-lowering drugs or to the diet. Reduced intestinal cholesterol absorption leads to increased cholesterol synthesis and increased expression of the LDL receptor. The effect on VLDL production is not known, and it is unclear whether bile formation and/or composition has changed. Serum triacylglycerol and HDL-cholesterol levels were unchanged in most studies. Various suggested mechanisms can be found in the literature to explain the mechanisms behind the cholesterol-lowering effects of plant sterols and stanols.

Plant sterols can displace cholesterol from mixed micelles. This is because they are more hydrophobic than cholesterol. Such a replacement reduces the concentration of micellar cholesterol. As a consequence, cholesterol absorption is lowered. It should also be noted here that plant sterols or stanols can reduce the rate of cholesterol esterification in enterocytes, and consequently the amount of cholesterol excreted via chylomicrons [2,17,18].

The review by Raguindin et al. in 2020 showed that the total content of phytosterols ranged from 35 mg/100 g to 68.2 mg/100 g in oats, while in buckwheat from 19 mg/100 g to 139 mg/100 g. The most abundant sterol in buckwheat and oats was sitosterol, constituting over 50–70% of all sterols, followed by campesterol [19]. In this study, the highest amount of sitostanol in the stool was found. However, in sprouts, diets, liver, and serum, they are significantly lower.

In seeds, control sprouts and probiotic-rich sprouts, 20.81 ± 1.93 mg/g lipids (33.71 mg/100 g product), 20.85 ± 1.88 mg/g lipids (50.46 mg/g product), 19.00 ± 2.18 mg/g lipids (45.79 mg/100 g of the product) were recorded, respectively. On the other hand, in the case of the tested material, both in seeds and sprouts, β-sitosterol was found in the highest amounts among the remaining compounds.

In a study by Yang et al in 2014, it was shown that in Tartary buckwheat, the following sterols, β-sitosterol, campesterol, had the largest share in the total amount of sterols [20].

It was noticed that the addition of the sprout lyophilisate to the atherogenic diet increased the total content of sterols in HFDCS diets (246.42 ± 5.57 μg/g lipids) and HFDPRS (394.64 ± 0.59 μg/g lipids) compared to the HFD diet (97.86 ± 0.97 μg/g lipids). There are no significant differences between the following HFD-S, HFDCS-S, HFDPRS-S groups in the total sterol content. By comparing the biochemical parameters, it can be observed that the HFDCS and HFDPRS groups had a significantly lower content of LDL-C cholesterol in the blood serum compared to the HFD group.

In a study by Liu et al in 2021, male Sprague Dawley rats were fed a high-fat diet for 6 weeks. They were then orally dosed with buckwheat protein (TBP) over a five-week period. The study showed that in serum, TBP supplement significantly lowered LDL-C levels and raised HDL-C levels [21].

Saccharomyces crevisiae var. *boulardii* may have the ability to alter cholesterol levels. The mechanism indicated by the researchers is the assimilation of cholesterol. It was shown in a study by Ryan et al. in 2015 that *Saccharomyces boulardii* removed cholesterol from laboratory culture media. This was carried out through its assimilation in yeast cells. Accordingly, *Saccharomyces cerevisiae* var. *boulardii* can assimilate intestinal cholesterol and subsequently alter serum cholesterol levels [22]. In the analyzed study, it can be noticed that rats fed a diet with the addition of control sprouts had a lower serum cholesterol content compared to the group fed an atherogenic diet with probiotic-rich sprouts.

Plant sterols are poorly absorbed in the intestine (0.4–3.5%), while plant stanols (0.02–0.3%) are absorbed even less. However, it is worth noting for comparison that the absorption of cholesterol ranges from 35 to 70%. There may be gender differences in this case. This is because the absorption of plant sterols and stanols in female rats appears to be higher than in male rats. Plant stanols can also reduce the absorption of plant sterols, and vice versa [2]. One of the reasons for the low absorption of plant stanols and sterols can mean plant sterols and stanols are weak esterified [23].

There is an indication in the works that the extent and rate of absorption of plant sterol or stanol depends on the length of the side chain and the presence of the five double bonds (saturation). Other factors may alter the intestinal absorption of sterols or stanols. Such factors may be mutations and polymorphisms in the ABCG5 or ABCG8 gene. An example is sitosterolemia, which is a rare autosomal recessive disease. It causes the accumulation of plant sterols and stanols and can lead to severe atherosclerosis at a very young age [2,24].

The enterohepatic metabolism of cholesterol and plant sterols is complex. In the lumen of the intestine, dietary cholesterol (and plant sterols) is reduced to free sterols by esterases and transferred to the micelles. Micelles are a mixture of bile salts, phospholipids, free sterols and some fatty acids. They interact with the apical membrane of enterocytes (a

process that has not been characterized at the molecular level). They allow sterols to enter enterocytes. Cholesterol is esterified with ACAT-2 and then incorporated into chylomicrons, which in turn are secreted at the basolateral surface into the lactealss. Eventually, they drain into the venous circulation. Lipoprotein lipase acts on the chylomicrons in the capillary beds of all organs. This allows the body to supply these tissues with triglycerides from the diet and some fat-soluble vitamins. The sterols in these molecules are not transferred and remain part of the remaining molecules. The particles are now recognized by the receptors in the liver and then purified. As a result, most of the sterols are delivered to the liver. Cholesterol enters the metabolic pool and can be repackaged into VLDL and excreted back into the circulation. When non-cholesterol sterols are excreted into the bile ducts, they return back to the intestinal lumen [25].

Unabsorbed cholesterol and plant stanols are excreted in the feces. Decreasing cholesterol absorption may increase its synthesis in the liver, and these amounts do not compensate for the losses; therefore, the level of total cholesterol and LDL fraction is lowered in the blood serum [3].

3.2. Fatty Acids

N-6 fatty acids, including linoleic acid (e.g., found in plant seed oils), are known to lower serum cholesterol, so it is generally recommended to partially replace dietary SFA with n-6 acids. With regard to PUFA, such dietary changes have been a hallmark of clinical trials. It is worth noting that PUFA-rich diets inhibit high-density lipoprotein (HDL), which protects against CHD, and also lowers LDL [26].

Diseases such as hyperlipidemia or atherosclerosis can be caused by a person's excessive consumption of high-fat and sugar products. This can happen by disrupting the lipid metabolism. Serum TG, TC, LDL-C, and HDL-C levels are major indicators that may reflect the body's lipid metabolism. An abnormal increase in blood lipids may lead to disease-threatening conditions [21].

In the study by Đurendic-Brenesel et al. in 2013, it was shown that supplementation with a mixture of buckwheat leaves and flowers significantly reduced body weight gain, plasma lipid concentration and atherogenic index in rats fed a high-fat diet [27].

In the publication by Raguindin et al. in 2021, it was indicated that the fatty acid content of oats and buckwheat was mainly composed of high levels of unsaturated fatty acids, i.e., oleic (C18:1) and linoleic (C18:2) acids; and palmitic acids (C16:0), stearic acid (C18:0) and linoleic acid (C18:3). Changes in the relative abundance of fatty acids with different anatomical parts of the plant; the effects of various environmental growth factors and genetic variants have been noted [19]. In the presented study, the highest amounts of linoleic acids (C18:2), unsaturated oleic (C18:1) and saturated palmitic (C16:0) acids were identified in both sprouts and seeds. Modification of seeds by adding a probiotic yeast strain influenced the content of the indicated acids in sprouts (it is higher in sprouts rich in probiotics than in control sprouts).

It was demonstrated in the study by Đurendic-Brenesel et al. in 2013 that a group of animals fed a high-fat diet with the addition of buckwheat leaves and flowers significantly increased the content of n-6 fatty acids and eicosapentaenoic acid (EPA). However, it reduced the content of saturated fatty acids (SFA) and oleic acid [27].

In the literature, there is a concept related to lipids of microbial origin, which are referred to as single cell oil (SCO). They are produced by microorganisms known as oleaginous microorganisms. They include molds, microalgae, bacteria and yeasts. They are defined as organisms capable of "producing and accumulating more than 20% of dry cell substance" [28–30]. Molska et al. in 2020 indicate that although *Saccharomyces cerevisiae* has not yet been identified in the literature as an oleaginous microorganism, this species is more often referred to as non-oil yeast. However, there are studies showing their effect on fatty acid production. *De novo* synthesis mechanisms stand out here, which consists of obtaining fat from acetyl-CoA and malonyl-CoA molecules [31]. The fatty acids synthesized by oleaginous microorganisms are primarily palmitic, stearic, myristic oleic, linoleic, linolenic

and palmitoleic acids [28,29]. In this work, this mainly applies to linoleic and linolenic acids, which, when comparing the control with probiotic-rich sprouts, shows an increase in its amount. In the experiment, the AIN-93M diet and atherogenic diets with the addition of common buckwheat sprouts were used. An increase in the amount of monoenoic and polyoic acids was observed in the HFDPRS group compared to the HFD group. Reduction in saturated fatty acids was also observed, compared to the HFDCS group.

The liver is the central organ, controlling lipid homeostasis through complex but precisely regulated signaling, biochemical and cellular pathways. Liver cells, the so-called hepatocytes, are the main parenchymal cells of the liver. They control metabolic functions in the liver, including triglyceride metabolism and biochemical functions. Fatty acids accumulate in the liver through de novo biosynthesis and uptake of liver cells from plasma. They are eliminated either by oxidation in the cell or by secretion into the plasma in triglyceride-rich very low density lipoproteins [32]. There were no significant differences between the atherogenic diet and the atherogenic diet with the addition of sprouts in blood biochemical parameters. Compared to the AIN control diet, a significant decrease in this parameter was observed.

In the livers of the HFDPRS-L group, a higher amount of C18: 0, C23: 0, C18: 3n-6 acids was observed, compared to the group fed with the control sprouts. In the serum, comparing the indicated groups, a greater amount of the following acids was found in the HFDPRS group: C16:0, C18:0, C18:1, C20:1, C18:2, C18:3n-3, C18:3n-6, C20:4n-6, C22:5, C22:6.

The indicated data coincide with the reports of other authors [19,23,32–34].

In the serum, when comparing the HFD-S, HFDCS-S and HFDPRS-S groups, a greater total amount of monoenoic and polyenoic acids was observed compared to the HFDCS group.

Dietary fatty acids are built into tissues and blood, and the fatty acid composition of these tissues is often used as biomarkers of fat intake. It is worth mentioning that both the amount and composition of fecal fatty acids reflect fat intake, intestinal fatty acid absorption and activity of colon bacteria [35].

In the faeces of animals from the HFDPRS-F group, a greater amount of excreted saturated fatty acids was found than in the HFD-F group.On the other hand, a lower amount of monoenoic and polyenic acids was observed, which may indicate a higher use of them by the body.

4. Materials and Methods

4.1. Animals

Thirty-two male Wistar albino rats (~8 weeks old) were used in the study. All the experimental procedures were approved by the local bioethics committee for animal studies (approval number 28/2017). Rats were housed on a 12-h light/dark cycle, thermostatically (20 °C ± 2) and with 55–65% humidity throughout the adaptation and experiment period. The mean weight of the rats was 188.7 ± 14.1 g. The experiment lasted 6 weeks.

4.1.1. Experimental Diets

The composition of the diets is presented in Table 6. The diets were developed on the basis of the AIN-93M diet modification [36]. Semi-synthetic diets consisted of wheat starch (Celiko, Poznań, Poland), potato starch (on potatoes from Iława, Poland), casein (from Murowana Goślina, Poland), soybean oil (ZPT Warsaw, Poland), sucrose (Diamant, Pfeifer & Langen Polska S.A., Poznań, Poland), choline (Sigma-Aldrich, Darmstadt, Germany), mineral mix (AIN-93M-MX) [36], vitamin mix (AIN-93-VX) [36] and choline (Sigma-Aldrich). In addition, the sprouts were added in the form of a lyophilisate to the HFDCS and HFDPRS diets. The diets were prepared by mixing all the ingredients.

Table 6. Composition of experimental diets (g/kg—AIN-93M, HFD diet; g/1.3kg—HFDCS, HFDPRS.

Component	AIN-93M	HFD [1]	HFDCS [2]	HFDPRS [3]
Caseine	140	140	140	140
Soybean oil (rapeseed)	40	40	40	40
Wheat starch	622.5	422.5	422.5	422.5
Potato starch	50	50	50	50
Lard	-	200	200	200
Saccharose	100	100	100	100
Mineral mix	35	35	35	35
Vitamin mix	10	10	10	10
Choline	2.5	2.5	2.5	2.5
Control sprouts	-	-	300	-
Probiotic-rich sprouts	-	-	-	300

[1] HFD—AIN93M + lard (high-fat diet). [2] HFDCS—high-fat diet + control sprouts. [3] HFDPRS—high-fat diet + probiotic-rich sprouts.

Buckwheat Seeds

Buckwheat seeds (*Fagopyrum esculentun* Moench) were purchased from PNOS S.A. in Ożarów Mazowiecki. The strain of *Saccharomyces cerevisiae* var. *boulardii* was grown for 48 h at 30 °C on malt agar. Then, the colonies were sterile picked and suspended in water. First, the seeds were disinfected with 1% (*v*/*v*) sodium hypochlorite (Sigma-Aldrich, St. Louis, MO, USA) for 10 min. They were then filtered and washed with distilled water until the pH was neutral. After reaching the expected pH, they were placed in an aqueous suspension of *Saccharomyces cerevisiae* var. *boulardii*, at a level of 1×10^7 mL^{-1} based on the OD value (probiotic-rich sprouts) or in distilled water for 4 h (control sprouts). The seeds were then placed in a growth chamber (SANYO MLR 350H) on Petri dishes (ϕ 125 mm) lined with absorbent paper, where they germinated in the dark for three days. Sprouting was run at 30 °C. After three days, sprouts were manually collected and rinsed with distilled water [31].

4.1.2. Experimental Design

Before starting the experiment, the animals were adapted to laboratory conditions. It lasted three days. During this period, animals had unlimited access to water and standard AIN-93M diet [36]. The animals were kept in stainless steel cages covered with non-metal glaze (throughout the adaptation and experimentation period).

After this period, the rats were randomized into four groups, each consisting of eight individuals. One of the groups was fed the AIN-93M diet; the next three groups were fed with the modified AIN-93M diet with the addition of lard in the amount of 200 g/kg for the HFD diet and 200 g/1.3 kg for the HFDCS and HFDPRS diets (Table 6) [36]. The addition of 600 g of sprouts, control and modified sprout lyophilisate, respectively, was included in the HFDCS and HFDPRS diets.

Each day, the animals were given a fresh portion of food and water, and any food and water residues from the previous day were removed. Diet and water consumption was monitored daily and the body weight of the rats was monitored weekly. The digestibility of the rats was determined during the experiment.

At the end of the experiment, the rats were weighed and then euthanized using carbon dioxide inhalation. During the section, the internal organs, including liver in this range, were removed, washed in saline, weighed and stored at −80 °C. Blood samples were collected after 12 h fast by cardiac puncture in serum-separated tubes to obtain serum.

4.1.3. Biochemical Parameters

Blood was collected by cardiac puncture into sodium heparin tubes to obtain whole blood for complete blood counts, and into tubes separated from the serum for the biochemical parameters. The coagulated blood was allowed to clot at room temperature for 30 min and then centrifuged for 15 min at 3600× *g*. The following biochemical parameters were

determined: glucose (GLU), triacylglycerols (TAG), activity of alanine transaminase (ALA), activity of aspartate transaminase (AST), total cholesterol (TCH), high-density lipoprotein cholesterol (HDL), non-HDL and protein C -reactive (CRP). Morphological index values were determined using a Sysmex K-1000 hematology analyzer (TAO Medical Electronics Co., Kobe, Japan) according to standard procedures. Serum glucose concentration was estimated by the glucose oxidase method. Serum total cholesterol and triglyceride levels were measured using commercial kits (Randox Laboratory Ltd., Crumlin, UK). The activity of liver enzymes, such as ALT and AST, was determined according to Dembińska-Kiec and Nastalski [37].

4.2. Determination of Crude Fats

Fat content in lyophilisates, seeds, controls and modified sprouts; liver, experimental diets and feces were determined using the Avanti Soxtec system (Model 2055 Manual Extraction Unit; Foss Tecator, Höganäs, Sweden), according to the AOAC Official Method 945.16 [38].

4.3. Fat Extraction

4.3.1. Fat Extraction from Freeze-Dried Seeds; the Freeze-Dried Control Sprouts; Freeze-Dried Sprouts Rich in Probiotics; Experimental Diet; Feces

Extraction of fat from freeze-dried seeds, freeze-dried control sprouts, probiotic-rich freeze-dried sprouts, experimental diet and feces were extracted according to the procedure described by Folch et al. (1957) [39]. The amount of fat was quantified and expressed as mg/g lipids (in buckwheat seeds and sprouts; liver; feces), μg/g lipids (in experimental diets) and μg/100 μL serum (in serum).

4.3.2. Hepatic-Fat Extraction

Three times ~1 g of tissue was collected from the scattered zones of the frozen left flap. The exact wet weight of each sample was determined after thawing and dehydration of excess moisture on the filter paper for 10 min at 25 °C. Total fat was extracted from liver samples using the Folch method with a slight modification [39,40]. Each sample (~1 g of tissue) was mechanically homogenized in 25 mL of chloroform-methanol (2:1) solution for 2 min. The homogenate was sonicated (VCX 750, Sonics and Materials Inc., Newtown, CT, USA) for 5 min at amplitude of 30%, with 5 s cycles on, 5 s off. The sonicated samples were then shaken overnight (12 h) at 25 °C. The wet mass from the solution was separated into a volumetric flask on the filter. The same amount of distilled water was then added as the amount of liquid in the flask. Sodium sulfate was placed on the filter to remove traces of residual water, and the contents were poured into a test tube. After separation of the layers, the layer of choloroform was transferred to another weighed test tube, the content of which was evaporated to dryness under liquid nitrogen. Then, the tubes were weighed to determine the amount of fat. The amount of fat was quantified.

4.3.3. Serum

The serum was stored at −80 °C. Fatty acids were extracted with the modified Folch method [39,41], briefly Folch reagent (2:1 chloroform:methanol), for lipid extraction from cells; butylated hydroxytoluene as an antioxidant and internal standard: deuterated myristic acid-d_{27} (d_{27}C14:0) in chloroform was added to the weighed samples and centrifuged. Extraction of the fat for the determination of sterols was performed in the same manner, except that the internal standard 5α-cholestane was added to the samples instead of the above-mentioned standard.

4.4. Analysis of the Fatty Acid Composition of: Freeze-Dried Seeds, Freeze-Dried Control Sprouts, Lyophilized Sprouts Rich in Probiotics, Experimental Diet, Liver, Feces

For the determination of fatty acids, they were converted into fatty acid methyl esters according to the method of AOCS Official Method Ce 2b-11 [42].

The heptadecanoic acid ester (0.25 mg/sample) was used as an internal standard. The samples were saponified with a 1 M solution of KOH in methanol. Then, they were incubated in a heating block for 20 min at 70 °C. The samples were cooled down and only then 5 mL of a 14% solution of BF3 in methanol was added to them. Again, the samples were incubated at 70 °C, for 20 min. After cooling, 2.5 mL of hexane was added to the samples. After mixing, 2.5 mL of water was added. After phase separation, 1 µL of hexane layer was taken and subjected to statistical analysis.

The gas chromatograph was a Trace 1300 with FID detector (Thermo Scientific, Waltham, MA, USA), while a SP TM-2560 capillary column (100 m × 0.25 mm × 0.2 µm) (Supelco, Bellefonte, PA, USA) was used for the analysis. The carrier gas was hydrogen (1.5 mL/min). The analysis was performed in splitless mode. The initial oven temperature was 160 °C for 1 min, and then it was increased 6 °C/min to 220 °C and it remained at this temperature for 17 min. The inlet and detector temperature were 240 °C. The retention times were compared to the retention times of 37 Component Fame Mix (Supelco, USA).

4.5. Analysis of Fatty Acid Composition of Serum

Approximately 2 mL of the lipid-containing chloroform phase was removed and derivatized with 0.5 mL of 0.5 M potassium hydroxide in methanol and 1 mL of boron trifluoride methylation by heating at 70 °C. Fatty acid methyl esters (FAME) were extracted into 4 mL of hexane and washed with 2 mL of distilled water. The hexane (organic) phase was then transferred to 2 mL vials. Then, the samples were analyzed by gas chromatography. The aforementioned analysis was carried out on a Hewlett-Packard 6890 gas chromatograph (Wilmington, DE, USA) equipped with a split/splitless injector and a flame ionization detector (FID). FAMEs were separated using a SelectFame column (50 m × 0.25 mm × 0.25 µm, Agilent Technologies, Santa Clara, CA, USA), identified by comparison with the available FAME standards (Supelco, Bellefonte, PA, USA). Fatty acid content was calculated by comparing the area of individual peaks with the area of the peak of the internal standard and recalculated on the basis of the sample weight Response factors (FID) for individual fatty acids compared to internal standards were taken as unity. The concentration of fatty acids was expressed quantitatively [41].

4.6. Analysis of the Composition of Sterols: Freeze-Dried Seeds, Freeze-Dried Control Sprouts, Lyophilized Sprouts Rich in Probiotics, Experimental Diet, Liver, Serum, Feces

The content of sterols and stanols was determined according to the AOCS Official Method Ch 6-91 [43]. For determinations, 50 mg of fat was taken and 50 µg of the internal standard 5α-cholestane was added, which was saponified with 1 M KOH in methanol. Extraction of the sterol fraction was performed using the hexane: MTBE system (1:1, *v*/*v*). The solvent was evaporated under nitrogen. After evaporation of the solvent, the residue was dissolved in 100 µL of pyridine and silylated with BSTFA reagent with 1 µ TMCS. The phytosterols were analyzed using a Hewlett-Packard 6890 gas chromatograph in splitless mode with an FID detector. A DB-35MS 30 m × 0.25 mm × 0.25 µm capillary column was used (J&W Scientific, Folsom, CA, USA). The injector and detector were kept at 300 °C; the oven temperature was initially 100 °C for 5 min, increasing at 25 °C/min to 250 °C and then at 3 °C/min to 290 °C. The final temperature was held for 20 min. The carrier gas was hydrogen and the flow rate was 1.5 mL/min. Sterols were identified by comparing their retention times with those of standards. The sterols were determined in duplicate. The concentration of fatty acids was expressed quantitatively.

4.7. Statistical Analysis

Statistical analysis of the data was performed using Statistica 10 (StatSoft, Tulsa, OK, USA). For statistical analysis, one-way analysis of variance and intergroup differences was used by Tukey's HSD post-hoc test with a significance level of $p < 0.05$. Significant differences were denoted with different superscript letters.

5. Conclusions

The study attempts to evaluate the effect of the addition of buckwheat sprouts modified with the addition of *Saccharomyces cerevisiae* var. *boulardii* to an atherogenic diet on the metabolism of sterols, stanols and fatty acids in rats. The analysis began with the analysis of the raw material, and then the diet, nutritional and biochemical parameters, as well as liver, serum and feces.

Based on the results, changes in the composition as well as the amounts of individual sterols, stanols and fatty acids were noticed. The addition of probiotic-rich sprouts with lyophilisate to the diet increased the amount of sterols compared to other atherogenic diets. There were 75.2% more sterols in the HFDPRS diet compared to the HFD diet.

It was noted that the group in which the modified sprouts were added to the diet had both a lower amount of cholesterol in the liver and feces compared to the rats fed a high-fat diet. The values are lower by 23.6% and 81.8%, respectively.

In the case of fatty acids, changes can be observed when comparing CS and PRS sprouts, including a reduction in the amount of saturated fatty acids in the group of modified sprouts, as well as a 35.5% increase in the amount of polyenic acids, which is of particular importance in the aspect of cardiovascular diseases.

Significant changes are observed in the range of fatty acids in the serum, liver, feces. There are statistically significant differences in the content of individual monoenoic and polyenic acids in the serum of rats fed the HFDPRS diet, compared to rats fed the HFDCS diet. It is influenced, among others, by acids such as C18:2, C20:4n-6, C22:6. They increase compared to the HFDCS group by 13.5%, 15.1% and 13.1%, respectively.

In the case of the HFDPRS group, there is a noticeably lower excretion of monoenoic and polyenic acids compared to the other studied groups. It is also worth mentioning here that the addition of sprouts to the atherogenic diet lowered the CRP index, i.e., acute phase proteins.

Referring to the hypotheses in the work, the following points can be made:

(1) It can be observed that the modification changed the content of individual compounds contained in the raw material, i.e., buckwheat. This is especially noticeable for the fatty acids C18:1, C18:2, C18:3n-3;
(2) Changes were noticed in the lipid profile by comparing the HFD and HFDPRS groups. In the HFDPRS group, a lower value of the non-HDL and LDL-C index was noticed.

It is advisable to conduct further research related to the metabolism of both sterols, stanols and fatty acids in the body in terms of the use of probiotic microorganisms. As it can be concluded from the obtained results, they indicate that the addition to the diet of the modified *F. esculentum* buckwheat seeds by the addition of the probiotic strain of the yeast *S. cerevisiae* may have a significant impact on the metabolism of the indicated components in the body.

The current trend of searching for new functional raw materials offers many opportunities for both technologists and dieticians. The presented research provides a new direction for the use of the raw material, buckwheat sprouts.

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12. DOROBEK NAUKOWY – WYBRANE OSIĄGNIĘCIA NAUKOWE

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